AN ABSTRACT OF THE THESIS OF

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Title: IRON AVAILABILITY FROM CORN TORTILLAS AND COOKED BEANS (PHASEOLUS VULGARIS); A STUDY ON THE EFFECT OF FIBER.

Abstract APPROVED: Dr. C. Jane Wyatt

Absorption of iron from foods has been reported to be inhibited by various factors including some diet components. Fiber from different sources has been observed to bind ferrous iron. This may be the reason for iron deficiencies observed in populations that consume diets high in non-digestible materials.

The effect of different levels of fiber from corn tortillas and cooked beans on iron availability in the rat was studied. Iron-depleted rats were fed test diets containing different levels of iron and fiber and the diets were tested for their hemoglobin repletion ability. Estimation of the neutral detergent fiber (NDF) in corn tortillas and cooked beans and the soluble and ionizable iron in the test foods and diets were also determined.

Corn tortillas contained 6.53% NDF. Raw beans contained 5.80% NDF and increase to 15.75% upon cooking. In cooked beans, 60.16% of the total iron is in the insoluble form while only 20.3% of the total iron in corn tortillas is insoluble.

The mean relative biological value (RBV) for the test diets was 55.0 ± 19.0% with values ranging from 40.41 ± 12.50% to 64.34 ± 11.10% compared to that of ferrous sulfate (100% available). Fiber or iron level had no significant effect on iron availability, however, a ratio

of 1.78 of corn tortilla to cooked beans showed the highest degree of iron availability.

Percent efficiency of the test diets to incorporate iron into hemoglobin ranged from 11.70 ± 1.4% for a diet containing 25 ppm iron and 15% NDF to 25.61 ± 6.10% for 35 ppm iron, 10% NDF diet. The control diet containing 30 ppm ferrous sulfate had a 43.0 ± 8.7% efficiency. Percent efficiency seems to show less variability in the determination of iron availability from foods than RBV.

A significant correlation ($p < 0.01$) between soluble iron at pH 1.35 in vitro and percent efficiency was observed. This method could be used to predict iron availability for different foods.

Iron Availability From Corn Tortillas and Cooked Beans (Phaseolus vulgaris); A Study on the Effect of Fiber

by

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TABLE OF CONTENTS

LIST OF TABLES

IRON AVAILABILITY FROM CORN TORTILLAS AND COOKED BEANS (PHASEOLUS VULGARIS); A STUDY ON THE EFFECT OF FIBER

INTRODUCTION

Iron deficiency is the most commonly recognized nutritional deficiency in developing countries as well as in affluent societies. It is particularly prevalent among infants and young children because during periods of rapid growth, iron needs are great. Most infant diets contain marginal supplies of iron (Goodhart and Shils, 1980).

Iron utilization is influenced by a number of physiological and non-physiological factors such as iron status of the subject, achylia, and pH of the gastrointestinal tract which may cause precipitation of iron (Cook et. al., 1964). Among the non-physiological factors that influence iron availability is the iron concentration in the food itself as well as its chemical form. That is, if it is soluble or insoluble or if it is in the ferric or ferrous state. Other food components like phytates, fiber, and phosphate have been reported to be possible inhibitors of iron absorption while other compounds, like ascorbic acid are reported to enhance iron absorption.

Corn tortillas along with cooked beans are the main staples in the Mexican diet. Corn tortillas are considered to be a rich source of fiber (Solomons et. al., 1979). Mexicans consume an average of 26 g of fiber per day (Reinhold and Garcia, 1979). That amount is high if compared with fiber consumption in developed countries. Fiber is thought to interfere with iron absorption by binding it and making it unavailable for absorption (Ismail-Beigi et al., 1977). More recently Reinhold et al., (1981), have shown that neutral detergent fiber (NDF)

from corn tortilla binds as much as 0.4 mg of iron per g of NDF at pH 6.45.

Legumes such as beans or peas are considered as good sources of iron (Bogert et al., 1973). The presence of fiber from corn tortillas in a diet of beans and corn tortillas may decrease iron absorption from beans. Miller (1978) found that incorporation of iron into hemoglobin was reduced when a corn diet was fed at two day intervals with a casein diet. Layrisse et al. in 1968 showed a decrease in iron absorption from veal after corn was introduced into the diet of experimental subjects.

The purpose of this work was to evaluate iron availability in rats from a combined diet of corn tortillas and cooked beans and to determine the effect of fiber on iron availability.

REVIEW OF LITERATURE

The role of iron in the body is almost exclusively confined to the process of respiration. It is a component of hemoglobin, myoglobin, cytochrome a, b, and P_{450}, a specialized microsomal cytochrome which plays a role in the metabolism of some drugs. Iron is also a component of the oxidative enzymes, catalase and peroxidase, as well as some metalloflavoproteins. The iron containing compounds involved in the respiratory process represent from 63.1 to 75.1% of the total body iron (National Research Council, 1979; Harper, 1973). Hemoglobin, myoglobin and cytochromes account for 25 to 35 mg iron/kg of body weight and more than 80% of the body iron is in the heme group of hemoglobin (Sjolin and Wranne, 1968; Smith and Rios, 1974). The storage compounds, ferritin and hemosiderin, account for 5 to 25 mg iron/kg body weight. When dietary iron supplies become inadequate, iron is mobilized from hemosiderin to ferritin, maintaining hemoglobin production as well as other iron containing compounds with known metabolic functions, such as cytochromes (Weinfeld, 1970; Hershko 1977). Although the exact mechanism by which iron is transported from the storage compounds is unknown, it may involve an oxidation and reduction process such as Fe (III) --- Fe (II) --- Fe (III) cycle (National Research Council, 1979).

Iron loss in men and women in the United States has been estimated to be 1 mg per day for the 80 kg. man or 12 µg/kg of body weight per day. In other countries the loss is 14 µg/kg of body weight per day (Green, et al., 1968), and 1.4 mg of iron per day for the 65 kg. menstruating female (Finch, 1959). The main iron loss is in the form of epithelial cells and through blood during the menstrual cycle in adult women.

During pregnancy, iron is transported to the fetus, thus increasing the iron requirement (Green et al., 1968).

The low iron requirement in men is due to the fact that 95% of the iron required for the production of red blood cells comes from the break down of senescent red blood cells. Therefore only 5% of the required iron must come from the diet (Finch, 1977). However this requirement does depend on the stage of growth since in growing children only 70% of the iron is recovered from the breakdown of senescent red blood cells, thus requiring 30% from the diet.

Iron needs are also influenced by the body's iron status. In an iron deficient state, iron loss is reduced to less than 50% but will increase to more than twice that level during a period of iron overload (National Research Council, 1979).

Iron absorption depends on the absorptive behavior of the intestinal mucosa, and the effect of various luminal factors such as gastric secretions. Absorption is usually reduced when gastric secretion is reduced or eliminated (Cook, et al., 1964). Action of pancreatic enzymes in the upper duodenum might well be expected to elaborate chelates, such as cysteine and histidine, which may improve iron availability (National Research Council, 1979). Iron absorption also depends on body iron stores, availability, and amount of iron in the diet (Dallman, et al., 1980; National Research Council, 1979). Some literature suggests iron is more readily absorbed in the ionic form, especially in the ferrous state rather than the ferric form which is absorbed less efficiently. This is probably due to the fact that iron must be reduced in order to be absorbed under normal physiological

conditions (Moore et al., 1944; Venkatachalam et al., 1956). Absorption of iron can occur both in the stomach and the intestines, however it seems that the greatest absorption occurs in the duodenum and progressively decreases in the more distal parts of the intestine. Colonic absorption has also been observed in the rat but the majority of iron absorption takes place in the duodenum and the upper part of the jejunum (Copp and Greenberg, 1946). Studies on the physical form of the iron in the stomach and duodenum indicate that iron is bound to macromolecules in the stomach but to micromolecules in the duodenum (Jacobs and Miles, 1969). Iron absorption is also influenced by the motility of the gastrointestinal tract. Retarded gastrointestinal motility increases iron absorption, whereas factors that decrease the transit time of the food tend to decrease iron absorption (Cammock et al., 1961; Magnusson, 1976).

Iron is absorbed directly into the blood stream rather than by way of the lymphatic system (Moore and Dubach, 1956). Some researchers have suggested that iron forms complexes with amino acids (e.g., cysteine and glycine) in the duodenum which enhances the passage of the bivalent iron through the cell membrane (Comar and Bronner, 1962).

Body iron stores appear to strongly influence the absorption capacity of the intestinal mucosa (Bothwell et al., 1958; Cook et al., 1969; Pirzio-Biroli and Finch, 1960). Low iron stores increase iron absorption whereas high iron stores result in diminished absorption (Cook et al., 1969). Hubers et al. in 1971 suggested that brush border cells of the mucosa villi might be one point of control. The nature of the absorptive process is unclear. Some suggest that iron absorption in the adult may

be regulated by the amount of iron received by the intestinal cell during its formation in the fetus (Conrad and Crosby, 1963; Weintraub, et al., 1964).

There are three stages of iron deficiency that can be defined: (1) the mildest form, characterized by depletion of iron stores; (2) the latent iron deficiency associated with reduction in serum iron concentration and elevated transferrin concentration with a corresponding drop in transferrin iron saturation to less than 15% (Bainton and Finch, 1964); and (3) an overt iron deficiency anemia in which restricted hemoglobin synthesis causes a decrease in the concentration of circulating hemoglobin (National Research Council, 1979). Worldwide and in the United States, iron deficiency is the most common nutritional problem. Estimates of prevalence are difficult to determine because of problems in defining what constitutes iron deficiency.

When values of transferrin saturation less than 15% were used as a criterion in detecting iron deficiency, it was found that 59.7 to 99% of pregnant women, 11.4 to 42.5% of non-pregnant women, and 0 to 11.4% men were anemic in selected populations in seven countries (WHO, 1968). In other studies in the United States, it was shown that 10% of the children from one to five years old were iron deficient (USDHEW, 1974).

Cook, et al., (1971) in a collaborative study in Latin America found 48% of the pregnant women, 21% of the non-pregnant women and 3% of the adult males to be iron deficient (transferrin saturation below 15%). In the same study 38.5% of the pregnant women, 17.3% of the non-pregnant women and 3.9% of the adult men were found to be anemic by using the WHO criterion (hemoglobin concentration below 11, 12 and 13 g/dl respectively).

Haghshenass et al., (1972) showed that in a typical village in Iran, 30% of the children, 24% of the females and 7% of the males over 16 years old had a hemoglobin concentration of 12 g/dl or lower, although the iron intake for boys 13 to 14 years old averaged 44.4 - 44.9 mg/day. Derman et al. in 1977 showed iron deficiency anemia was common among Indian populations but suggested in this case it may be due to the low iron levels in the diet.

Sources of Iron

There are two major sources of iron in the diet, heme and non-heme iron. Non-heme iron is mainly provided by vegetables while heme iron is supplied from animal sources (Hallberg and Bjorn-Rasmussen, 1972; Layrisse et al., 1969). Iron availability from heme sources is high in contrast to non-heme iron. Availability of dietary iron is influenced by many factors including other food constituents.

Martinez-Torres et al. (1974) found absorption of iron from veal was 20% in normal subjects but if the individual was iron deficient absorption increased. The addition of maize reduced veal iron absorption but increased maize iron absorption. Iron from breast milk is considered to be 49% available which is high compared with 10 to 12% availability in unfortified cow's milk formulas and 4% from fortified milk formulas (Saarinen and Shmes, 1977; Rios et. al., 1975). The high bioavailability of breast milk iron is markedly decreased when solid food is introduced in the diet suggesting an inhibitory effect of vegetable foods (Rios et al., 1975).

Layrisse and Martinez-Torres, (1971) in a human study found 7% of the iron from soybeans was absorbed compared with 1 and 3.5% from rice and

corn respectively. In a study where normal young males were made anemic by phlebotomy, there was no correlation between the amount of iron absorbed and the amount of iron ingested. There was a pronounced individual variation in the mean dietary absorption ranging from 1.9 to 5.0 mg of iron. Iron absorption was not influenced by high or low intakes of animal products, cereal or vegetables. A negative correlation was found between iron absorption and the amount of fecal dry matter (Olszon et al., 1978).

Layrisse, et al. in 1968 found normal subjects absorbed 2% of the iron from black beans and corn. They also observed a slight reduction in iron absorption from veal when it was fed with black beans and corn. Derman et al. in 1977 reported absorption of iron from maize porridge was very small (3.8%) being further reduced to 2.1% when tea was included in the meal. In another study Ashworth et al. in 1973 showed the mean absorption of iron from maize was 4.3% and 9.4% from baked soybeans. The poor availability of iron from maize is probably the most important cause of iron deficiency anemia in Jamaican children.

Ashworth and March in 1973 in another study reported the addition of maize to a meal of dry skim milk supplemented with iron sulfate caused a decrease in iron absorption from 9.5 to 6.3% although absorption was higher than when maize was eaten alone.

Sheila and Warner in 1968 found the absorption of iron from labeled bread (^{59}Fe) to be 6.7% as compared with 42% for the standard, 5 mg of ferrous iron. However absorption was enhanced with the addition of orange juice to the diet.

As can be seen iron absorption is rather complex and is influenced by iron status of the subject as well as the form of iron and type of

foods in the diet. A large number of compounds have been suggested to interfere with iron absorption by different mechanisms. Some of the compounds that may influence iron absorption are ascorbic acid, phytate, oxalates, and fiber.

Phytates and Mineral Absorption

It has been observed that phytic acid is capable of forming insoluble complexes with different metals (Oberleas et al., 1966). Since phytic acid is found in plants it is considered as an antinutritional factor present in many foods.

Haghshenass et al. (1972) attributed the occurrence of iron deficiency anemia in an Iranian population to high levels of phytate in unleavened wholemeal wheat bread. Bjorn-Rasmussen in 1974 suggested the reduction in iron absorption from bread in man as the concentration of bran increased was due to an increase in phytates in the bran. However, Ismail-Beigi et al. (1977) reported the removal of phytates had no effect on the metal binding capacity of wheat bran at pH 6.5 and 6.8.

In a human study Morris et al. (1980) found the phytates present in 36 g of wheat bran consumed each day did not exert a detrimental effect on trace mineral nutrition. Liebman and Drukell in 1979 by using the rat found the consumption of six different levels of phytate (48 to 158.8 mg/day) did not effect hemoglobin repletion or total iron in the liver. These investigators concluded phytate in the diet does not have an effect on iron absorption. Reinhold et al. in 1975 found mineral availability in wholemeal bread was enhanced when phytates were removed. Fiber in the wholemeal bread also binds minerals. This fact explains in part the decrease in availability of dietary iron and zinc in wholemeal wheat bread

as observed by Ismail-Beigi, et al. (1977). Welch and Van Campen (1975) found the availability of ^{59}Fe added to soy beans was not correlated with the phytate content in the beans.

The presence of phytates in the diet does not totally explain the observed mineral abnormalities in subjects where cereal and vegetables are the main staples in their diets. Hence, fiber has been suggested to interact with minerals and influence their availability (Solomons et al., 1979; Kelsay et al., 1978; Sandstead et al., 1978).

Fiber a Problem of Definition

Although it has been suggested that fiber plays a role in nutrition there is not agreement in its definition. Fiber is largely defined by the method used for its determination and fiber contains a variety of compounds with known properties (Cummings, 1978).

Originally fiber was defined as the indigestible part of foods and fiber was analyzed to identify the non-nutritive value of foodstuffs (Cummings, 1976). As the knowledge of fiber components increased as well as its physical and chemical properties a better definition was needed.

Van Soest (1973) reviewed the criticisms of using the term crude fiber. In 1972, Trowell introduced the term dietary fiber which was defined as that portion of the plant cell wall resistant to degradation by endogenous secretions of the human gastrointestinal tract. In 1978, Southgate et al., confirmed this definition. Saunders et al. (1980) believed that non-digestible fractions of proteins, starch, lipids, minerals, sugars and other non-metabolized compounds such as products of the Maillard reaction are part of the dietary fiber complex.

It has been shown by Saunders and Hautala (1979) that crude fiber, neutral detergent fiber, and in vitro dietary fiber correlate with in vivo (rats) dietary fiber in wheat foods. However, it seems from the available literature, more studies need to be conducted with other foods.

Fiber and Its Implication in Human Nutrition

The incidence of a variety of diseases or disorders, like cancer of the colon, low levels of insulin production and high blood glucose levels, have been associated with low consumption of fiber in the developed countries. In African populations where consumption of fiber is high, the incidence of these disorders is low (Kelsay et. al., 1978).

Jenkins et. al. (1975) reported the addition of 36 g of wheat fiber increased the daily excretion of bile acids and neutral steroids, but blood cholesterol and triglycerides were unchanged. On the other hand, Farrell et. al. in 1978 found a significant decline in plasma cholesterol with the addition of fiber. Kritchevsky and Story (1974) found different non-nutritive fibers capable of binding sodium taurocholate and glycocholate. Alfalfa non-nutritive fiber bound more bile salts than any other non-nutritive fiber tested, suggesting the binding of salts as an additional mechanism by which alfalfa may function in preventing hypercholesteremia.

One of the most discussed properties of fiber is its binding capacity with metals. Bjorn-Rasmussen (1974) found the addition of 0.3 to 40% bran reduced significantly the absorption of iron in humans. He suggested that the addition of approximately 7% bran to wheat bread decreased the iron absorption by a factor of two.

Cummings (1978) found an increase in mineral excretion with an increase in dietary fiber intake. Sandstead et al. (1978) reported the addition of 26 g of soft wheat bran (American Association of Cereal Chemists) to a basal diet appeared to decrease the retention of zinc in male volunteers, but apparently improved copper balances. On the other hand, the addition of corn bran in the same study had little effect on copper and zinc balance. The effect of fiber on iron was unclear.

Kelsay (1978) found a negative balance of calcium and magnesium with high fiber diets in humans, although iron balance was not significantly influenced by fiber. Kelsay et al. in 1979 suggested that the no effect of fiber on iron balance in humans may have been due to a high intake of iron, or a high level of ascorbic acid, or meat products in the diet.

Solomon et al. (1979) suggested the high consumption of fiber from corn tortillas by rural populations of Guatemala may affect zinc absorption. Ismail-Beigi et al. (1977) found the binding of zinc in wholemeal bread, dephytenized tanok, and cellulose is pH dependent. Maximum binding occurred at pH 6.5 - 7.5. In wheat bran at pH 6.5 and 6.8, 72% of the iron (0.5 mg/ml) and 82.5% of the zinc (1.43 mg/ml) were bound. Bran and hemicellulose exhibited a high iron binding capacity and the percent of bound iron decreased as the iron concentration increased. Kelsay (1978) found an increase in the excretion of zinc, phosphorus, and iron as cellulose was added to human diets. Fetzer et al. in 1979 found that hemicellulose and pectin decreased the availability of zinc, copper, and magnesium to a greater extent than did cellulose. Reinhold et al. (1975) suggested that fiber largely determines the availability of

bivalent metals in bread. Absorption of metals takes place in the small intestine. Although fiber is degraded by bacteria in the large intestine resulting in the release of metals, metals cannot be absorbed in this region. Thereby, metals are excreted in the feces.

James et al. in 1978 reported that dietary fiber from plants low in phytates bound calcium in proportion to its uronic acid content. The bound calcium would not be absorbed. They also pointed out that the ability to maintain calcium balances on high fiber diets may depend on the adaptive capacity of the colon for absorption of calcium. More recently Reinhold et al. (1981) found ferrous iron firmly bound to neutral detergent fiber fractions from wheat or maize. The amount of iron bound depends upon iron concentration, pH, quantity of fiber as well as the presence of binding inhibitors.

MATERIALS AND METHODS

Experimental Design

The design of the experiment is as follows:

I. Purpose: To evaluate the effects of fiber from corn tortilla and cooked beans on the bioavailability of iron in the rat.

II. Experimental Animal: Male Long Evans rats, 28 days old (3 animals per group).

III. Experimental groups: A 3 x 3 factorial design was used for the test diets, three levels of neutral detergent fiber (NDF) (6.5, 10, 15%) and three levels of total iron (25, 30, 35 ppm) and two positive controls (25 and 35 ppm iron as ferrous sulfate) were fed during the repletion period.

IV. Experiment period:

Depletion: 51 days on iron free diet.

Repletion: 14 days on test diets.

V. Parameters Measured:

Depletion: (a) Body weight

(b) Hemoglobin concentration

Repletion: (a) Body weight

(b) Feed consumption

(c) Hemoglobin concentration

(d) Liver weight

(e) Liver iron

Analytical: (a) Total iron in diet

(b) Percent NDF in corn tortillas and cooked beans

(c) Ionizable iron in cooked beans and corn tortillas

(d) Soluble iron in corn tortillas and cooked beans.

Diets

Diets were formulated using corn tortillas and cooked beans to provide 66.49 ± 1.98% of total calories. The experimental diets had three levels of neutral detergent fiber (NDF) (6.5, 10, 15%) and three levels of iron (25, 30 and 35 ppm). The dietary ingredients of the various diets are listed in Table I.

The corn tortillas and the beans were obtained from Mexico. The corn tortillas were sun dried and ground to pass a 18 mesh screen. The powder was stored at -10°F until blended into the diet. The beans were soaked overnight in tap water at room temperature (approximately three to one ratio), and cooked in an open vessel at boiling temperatures until done (approximately two hours). The cooked beans and the liquid were freeze dried and ground to pass a 20 mesh screen[1]. The remaining ingredients used in the diet were all purchased from United States Biochemical Corp (Cleveland, Ohio).

The amount of cooked beans in each of the test diets was held constant. The different NDF fiber levels of 6.5, 10 and 15% were achieved by increasing the amount of corn tortilla and by the addition of Cellufill. The three different iron levels were obtained by supplementing the endogenous iron levels of the corn tortillas and the cooked beans with ferrous sulfate heptahydrate to the desired level. Three control diets at 0, 25 and 35 ppm of ferrous sulfate were used in the study. After complete mixing of the diets, the material was placed in plastic bags

[1]Oregon Freeze Dry Inc., Albany Ore. 97321.

Table 1. Percent of Ingredients in Experimental Diets.

Diet	Corn Tortilla	Cooked Beans	Casein (Vitamin Free)	Glucose Monohydrate	Corn Oil	Vitamin[1] Mix	Cellufil	Mineral[2] Mix	$FeSO_4 \cdot 7H_2O$
BASAL 0	-	-	20	69.6	5.0	3.5	-	2.20	no
BASAL 25	-	-	20	69.6	5.0	3.5	-	2.20	yes
BASAL 35	-	-	20	69.6	5.0	3.5	-	2.20	yes
15 NDF 35[3]	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	no
15 NDF 30	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	yes
15 NDF 25	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	yes
10 NDF 35	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	no
10 NDF 30	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	yes
10 NDF 25	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	yes
6.5 NDF 35	50	25.2	4.18	11.82	3.51	3.5	-	2.20	no
6.5 NDF 30	50	25.2	4.18	11.82	3.51	3.5	-	2.20	yes
6.5 NDF 25	50	25.2	4.18	11.82	3.51	3.5	-	2.20	yes

[1] AIN vitamin mixture 76, U.S. Biochemical Corp., Cleveland, Ohio 44122.

[2] AIN Mineral mix 76, U.S. Biochemical Corp., Cleveland, Ohio 44122.

[3] 15 NDF 35 = % NDF (neutral detergent fiber) ppm iron.

and held at -10°F until fed.

Animals and Housing

Weanling male Long Evans rats were obtained from Charles River Breeding Laboratories (North Wilmington, Mass). Upon receipt, the animals were 28 days old. The rats were housed individually in stainless steel cages with wire mesh floors. Lighting was automatically controlled to provide twelve hours of light and twelve hours of darkness. Feed (in aluminum cups) and deionized water (in rubber stopper glass bottles) were supplied ad libitum. Final weight gain as well as total feed consumption were recorded.

Iron Bioavailability

The rats were fed an iron free diet for 51 days. At the end of this period (depletion period) the hemoglobin concentration in the blood was measured. The blood drawing was taken from the tip of the tail.

The anemic animals were divided into groups of three animals each in such a manner that the mean hemoglobin concentration was 9.91 g/dl. One group of rats, as a control group, was maintained on the iron free diet. The remaining animals were placed on the test diets. After a 14 day repletion period the hemoglobin concentration was again determined (Fritz and Pla, 1972). At the termination of the experimental feeding period the animals were killed by placing in a CO_2 chamber and the livers were removed. The livers were placed in vials and frozen immediately for total iron analysis.

Hemoglobin Determination

Animals were subjected to blood drawings at the end of the depletion

and repletion periods. The animals were immobilized in a stainless steel animal holder, the tip of the tail was cut and blood samples were collected by using 20 µl heparinized micropipets (in some cases the tail was soaked in warm water to help blood drawing). The blood was analyzed for hemoglobin by the cyanomethemoglobin method (Crosby et al., 1954) using a Bausch and Lomb Spectronic 20 Colorimeter. Drabkin's solution as well as the hemoglobin standard were obtained from Sigma Chemical Co. (St. Louis, MO.).

Hemoglobin concentration was obtained from a standard curve which ranged from 0 to 18 g/100 ml of blood and by using the following linear regression line: $Y = 0.2578 + 36.05 X$, where Y = hemoglobin concentration in g/100 ml of whole blood and X = absorbance at 540 nm. The samples were analyzed by adding 20 µl of whole blood to the tube containing 5 ml of Drabkin's solution, rinsing the micropipette several times, thoroughly mixing the solution, allowing it to stand 20 minutes at room temperature and recording the absorbance at 540 nm. Drabkin's solution was used as a blank.

Analytical Procedures

Moisture

Percent moisture of the diets, as well as of the corn tortillas and cooked beans powders was determined after drying to a constant weight in a vacuum oven at 80°C (AOAC, 1980).

Protein Determination

Protein determination in corn tortillas and cooked beans was done

by the macro-Kjeldahl procedure (AOAC, 1980). Protein levels are reported as organic nitrogen x 6.25.

Crude Fat

Crude fat in cooked beans and corn tortillas was measured according to the Association of Official Analytical Chemists procedure (1980).

Total Iron Content

Total iron content of the different materials was determined by a procedure similar to that described by Simpson and Blay (1966). Two gr. of sample were weighed into a 100 ml volumetric flask to which 40 ml of concentrated HCl acid were added. The flask was then heated to boiling for 30 minutes. The samples were cooled, made to volume with deionized water and filtered through filter paper (Whatman No. 1). The clarified solution was analyzed in an Atomic Absorption Spectrophotometer (Perkin-Elmer 303) using 40% HCl standards.

Soluble and Ionizable Iron

The soluble and ionizable iron content of the corn tortilla, cooked beans and all the test diets were determined using the method of Narasingas et al. (1979). Two grams of dry sample powder, 25 ml of pepsin-HCl solution (0.5% pepsin solution in 0.1N HCl) were added. The pH of the solution was adjusted to 1.35 by the addition of 4N HCl. The solutions were incubated at 37°C for 90 min. in a water bath shaker. After the incubation period the samples were centrifuged for 45 min. at 4000 RPM and 10°C. Finally the supernatant was filtered through Whatman No. 44 filter paper. The soluble iron was determined in the supernatant

by atomic absorption spectroscopy.

The ionizable iron was measured according to the procedure described by Henry et al. (1974). The ionizable iron is converted to the ferrous form complexed with bathophenantroline sulfonate to form a colored compound whose absorbance is measured spectophotometrically at 535 nm. It was necessary to modify the procedure by adding an equal volume of chloroform to the supernatant to clarify the solution. The solution was thoroughly mixed, centrifuged for 15 minutes at 4000 rpm, the upper layer was extracted and used for the ionizable iron determination.

Neutral Detergent Fiber (NDF)

Determination of the NDF fraction in raw and cooked beans and corn tortillas was done by using the method of Goering and Van Soest as modified by Rheinhold and Garcia (1979). A 0.5 g sample was weighed into a 250 ml beaker, 20 ml of water and 2.5 ml of a 2% amylase solution (Amylase Type III) was added. The solution was heated to boiling until two thirds of the volume had evaporated. Fifty ml cold NDF reagent[1] was added and the solution gently boiled for 30 additional minutes. Another 50 ml of NDF reagent and 2.5 ml of the amylase solution added, and the solution reheated for 30 additional min. Contents of the beaker were transferred to a tared 50 ml Gooch crucible (W_1), filtered under low vacuum washing twice with hot water and twice with acetone, dried over-

[1]To 1 liter of distilled water add: 30 g sodium dodecyl sulfate, USP; 18.61 g disodium dihydrogen ethylenediaminetetraacetic dihydrate, reagent grade; 4.56 g disodium hydrogen phosphate, anhydrous, reagent grade; 6.81 g sodium borate decahydrate, reagent grade and 10 ml 2-ethoxyethanol, purified grade, agitate to dissolve. Check pH and adjust to 6.9-7.1

night at 100°C, cooled and weighed (W_2). The percent NDF calculated according to the following formula and corrected for its ash content.

$$\% \text{ NDF} = \frac{W_2 - W_1}{\text{g sample}} \times 100$$

Acid Detergent Fiber (ADF)

The ADF fraction in cooked beans was determined by the method of Goering and Van Soest (1970). To one gram of dry bean powder in a 300 ml round bottom flask, add 100 ml of ADF solution[2], reflux for 60 min., after which the solution is transferred to a tared 50 ml Gooch crucible (W_1). Sample washed twice with hot water and twice with acetone under low vacuum, dried overnight at 100°C, cooled in a desiccator and weighed (W_2). The percent of ADF was calculated as follows:

$$\% \text{ ADF} = \frac{W_2 - W_1}{\text{g sample}} \times 100$$

Inhibition of the Maillard Reaction

Raw beans (30 g) were washed with distilled water and soaked overnight in a 0.002% solution of sodium metabisulphite. After soaking the beans were cooked by boiling for one hour. An additional 100 ml of 0.02% sodium metabisulphite was added during cooking. The cooked beans were then dried and ground to pass a 120 mesh screen.

Protein Extraction

Beans proteins were extracted from raw beans by washing the raw

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[2]ADF solution; dissolve 20 g technical grade cetyltrimethyl ammonium bromide in 1 N H_2SO_4 (1 liter).

powder bean with 2% NaCl. To 10 g of the powder, 150 ml of distilled water was added, the slurry was mixed for 30 min at room temperature and then centrifuged for 20 min at 9000 rpm and 5 C. The supernatant was discarded. The residue was then washed twice with 100 ml of 2% NaCl solution and once with 100 ml of distilled water. The residue was cooked in a microwave oven for one minute, dried overnight and ground to pass a 120 mesh screen.

Leucoanthocyanin Determination

Extraction and identification of leucoanthocyanins in the NDF fraction of cooked beans was accomplished by using a solution of 25 ml concentrated HCl in 500 ml of n-butanol. The NDF fraction (125 mg) were placed into a test tube and 8 ml of the n-butanol HCl solution added. The tubes were mixed thoroughly and heated to 90 C for 40 min in a water bath. The tubes were cooled, centrifuged in a clinical centrfuge, and absorbance at 550 nm of the supernatant was determined.

Iodine Test

The presence of starch material in the NDF fraction was determined by placing a portion of the NDF fraction in a test tube containing 5 ml of distilled water and heating for 15 min in a boiling water bath. A few drops of iodine solution (2%) were added. Starch will give a characteristic blue color.

Statistical Analysis

The data were statistically analyzed by analyses of variance (ANOVA), correlation coefficient and multiple regression according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSIONS

Composition of Test Diet Ingredients

The protein, crude fat, dry matter, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content of the test ingredients are shown in Table II.

The protein concentration (Kjeldahl N x 6.25) of 27.88% in cooked beans is in agreement with values reported by Sgarbieri *et al*. (1979) and with those reported by Kon (1979). This value is higher than the 18.60 to 23.0% range reported by Elias *et al*. (1979) in Guatemalan varieties of beans (*Phaseolus vulgaris*). The protein concentration in corn tortilla was found to be 11.60%. This value is high compared with a value of 10.50% protein reported by Robson *et al*. (1976) for corn, however some variation is expected to occur with the type and variety of corn used in making the tortilla.

Crude fat percentages of 1.76% for cooked beans agree with values reported by Watt and Merrill (1963). No values have been reported for the crude fat content in corn tortillas.

The level of NDF in corn tortillas was 6.53%, which is similar to the level previously reported by Reinhold and Garcia (1979). A concentration of 5.80% NDF was observed in the raw beans. After cooking the NDF level increased to 15.75%. Such increase is attributed to the production of insoluble proteins and other non-digestible products such as those produced by the Maillard reaction (melanoidins type products) during cooking (Van Soest, 1965). Saunders and Hautala (1979) believe melanoidins as well as other non-digestible compounds should be included in dietary fiber values. Whether or not this NDF value in cooked beans really

Table II. Composition of Corn Tortillas, Raw and Cooked Beans (% on a dry wt basis).

Item	Protein	Crude Fat	Dry Matter	NDF[1]	ADF[2]
Raw beans	-	1.37	-	5.80	5.06
Cooked beans	27.38	1.76	98.80	15.75	6.96
Corn tortillas	11.60	1.89	90.11	6.53	-

[1]NDF - neutral detergent fiber

[2]ADF - acid detergent fiber

represents the amount of undigestible material present, remains to be determined.

When the Maillard reaction was inhibited during the cooking process the NDF value was decreased to 10.47%. If the proteins were extracted before cooking the NDF value was 12.44%. Although these values are lower than the value reported for total NDF, it is difficult to conclude with certainty that the Maillard reaction and the production of insoluble proteins during cooking are really responsible for the increase in the NDF levels in cooked beans. Later experiments in which different enzyme concentrations (amylase) as well as lower temperatures and longer periods of digestion (from 1 to 24 hr. at 40^{o}C) were used showed a range of values from 9.12% to 10.50% with an average of 9.88% suggesting that the conditions during starch digestion are critical and possibly the high values could be due to incomplete digestion even though the samples were shown to be free of starch by the iodine test after digestion.

Data obtained does indicate the insoluble protein fraction as well as Maillard reaction products do contribute to the NDF values obtained since the NDF fraction of cooked beans contained as much as 1.16% total nitrogen. Other compounds could also be involved in this increase such as pigments. Leucoanthcyanins are thought to be one of these compounds. Cooked beans were found to contain leucoanthocyanins (7.70 units absorbance at 550 nm/gr of dry sample) when they were extracted with a butanol-HCl solution (25 ml of 36% concentrated HCl in 500 ml of n-butanol). Phenolic type products were also detected in both cooked and raw NDF fractions. These compounds could contribute to the observed increases in the NDF fraction of cooked beans.

An increase in the ADF fraction was also observed. This fraction contains cellulose and lignin so that the increase in NDF cannot be totally attributed to the presence of hemicellulose type compounds.

The NDF fraction of cooked beans may represent an increase in the total fiber consumption in populations in which beans are one of the main staples in their diets. Reinhold and Garcia in 1979 reported the fiber level of Mexican diets to be 26 g/day. The value may even be higher, due to the contribution of cooked beans, however the nutritional implication of this increase has not been determined. Monte and Maga (1980) reported a similar increase in the insoluble matter after pinto beans were cooked. They reported an increase of approximately 20% in insoluble matter. Similar effects have been observed by Matthee and Appledorf (1978) in legumes.

Forms of Iron in the Diet

The total, soluble, and ionizable iron in cooked beans and corn tortilla as well as in the experimental diets are shown in Table III. A total iron level of 5.12 mg/100 g in cooked beans compares favorably with values reported by other researchers. Sgarbieri *et al*. in 1979, reported a value of 5.59 mg/100 g and Augustin *et al*. in 1980 a value of 5.84 mg/100 g. One might expect some variability among different varieties and growing conditions.

The total iron value of 2.66 mg/100 g found in corn tortillas agrees with the value of 2.8 mg/100 g reported in maize by Robson *et al*. (1976). Layrisse *et al*. in 1968 reported 2.2 mg/100 g of total iron in corn and Hunt *et al*. (1978) reports a value in corn tortillas made in Los Angeles,

Table III. Form of Iron in Corn Tortillas and Cooked Beans. (mg/100 g dry wt.).

SAMPLE	TOTAL Fe (A)	SOLUBLE Fe[1] (B)	IONIZABLE Fe[1] (C)	INSOLUBLE Fe[2] (A - B)
Corn tortilla	2.66	2.12	1.84	0.54
Cooked beans	5.12	2.04	1.14	3.08
15 NDF 35[3]	5.51	1.85	1.56	3.66
15 NDF 30	5.09	1.70	1.18	3.38
15 NDF 25	3.93	1.57	0.82	2.26
10 NDF 35	5.02	1.55	1.19	3.47
10 NDF 30	4.78	1.31	0.97	3.47
10 NDF 25	4.18	1.34	0.87	2.84
6.5 NDF 35	4.89	1.14	0.88	3.75
6.5 NDF 30	4.85	1.02	0.79	3.83
6.5 NDF 25	3.81	1.52	1.24	2.29
0 NDF 35	3.64	1.81	0.64	1.83
0 NDF 30	1.57	1.53	0.44	0.04
0 NDF 0	1.43	1.09	0.53	0.34

[1]Determined at pH 1.35

[2]Difference between soluble at pH 1.35 and total iron

[3]15 NDF 35 = 15% NDF 35 ppm Fe

California almost double from previous work. This variability could be due mainly to differences in varieties and methods of preparation rather than to a disagreement in the results obtained.

The soluble iron in cooked beans was 2.04 mg/100 g. Wallis and Jaffee in 1977 found a range of 0.47 to 3.39 mg/100 g in beans. Cooked beans contain 1.14 mg/100 g of ionizable iron and no values have been reported in the literature for ionizable iron in cooked beans.

Corn tortillas contain about half of the total amount of iron present in beans, however the majority of this iron is in the soluble and ionizable state, 2.12 mg/100 g and 1.84 mg/100 g respectively. It has been pointed out recently that in addition to the total iron content of food, the form of that iron is also important in terms of availability (Lee and Clydesdale, 1979; Narasingas et al. 1979). Thus it appears that even though the level of iron is low in corn tortillas it may be readily utilized.

Iron Bioavailability

Two methods were used to calculate iron availability from the experimental diets. The first relative biological value (RBV) is a modification of the method by Pla and Fritz (1971) which takes into consideration body weight (Chao-Lo, 1979). The RBV evaluates the availability of the iron in the test diet by comparing its ability to replenish hemoglobin levels against that of ferrous sulfate. The ratio of the test iron to iron from ferrous sulfate is computed to produce an equivalent hematological response. Ferrous sulfate is considered to be 100% available.

The product of the rats body weight times hemoglobin concentration in g/100 ml of whole blood attained at the end of the repletion period

was plotted against the amount of iron (as ferrous sulfate) present in the basal diet. (Basal -0, Basal -25, and Basal -35). The following equation was used in making these calculations.

$$g\ Fe\ (as\ FeSO_4 \cdot 7H_2O) = -11.45 + .00634 \times (Final\ Hb \times Body\ Weight)$$

The values for the product, Final Hb x Body Weight for the experimental diets were substituted into the formula to calculate the quantity of ferrous sulfate that gave the same response as that obtained with the concentration of iron present in the experimental diet. The relative biological value (RBV) was then estimated as follows:

$$RBV = \frac{100 \times g\ FeSO_4}{\text{Iron conc. in diet}}$$ that gave same response in Hb value

It is worth noting that this method does not take into consideration differences in feed consumption among rats.

The second method gives us a more realistic estimation of iron availability since it takes into consideration feed consumption. It consists of calculating the ratio between the iron incorporated into hemoglobin and the amount of iron consumed from the diet through the repletion period and expressed as a percent efficiency of iron utilization.

$$\%\ Efficiency = \frac{\text{Final mg of Hb Fe} - \text{Initial mg of Hb Fe}}{\text{mg of Fe consumed}} \times 100$$

The amount of hemoglobin iron (Hb Fe) was calculated taking into consideration that 6.5% of the rats body weight is blood and that hemoglobin contains 3.4 mg of Fe/g.

Relative Biological Value (RBV)

The relative biological value (RBV) for the experimental diets is shown in Table IV. Considering that the RBV value for the ferrous sulfate is 100, the values for the test diets range from 40.41 ± 12.50 to 64.34 ± 11.10, with a mean RBV value of 55.00 ± 19.10

Variation was observed within groups and between groups as can be seen by the large standard deviations. In general higher RBV values were obtained when the rats were fed a diet containing 10% fiber. The greatest variation was experienced with the 15% fiber test diet. Variations within groups are attributed to the neutral animal response as well as to the differences in feed consumption.

Percent Efficiency for Iron Repletion of Test Diets

The percent efficiency for the experimental and the basal diets are shown in Table V. The values range from 13.79% ± 2.8 for the lowest efficiency to 25.65% ± 6.1 for the highest percent efficiency, with a mean of 17.78%. The percent efficiency for the diet containing 35 and 25 ppm ferrous sulfate was 47.67 ± 8.1 and 43.0% ± 8.7 and 0.42% ± 1.1 for the iron free diet. Sgarbieri *et al*. in 1979 had previously reported a 30% efficiency for ferrous sulfate containing diets.

Less variability in efficiency to restore hemoglobin iron was observed between animal groups and individual animals as compared with the RBV values as can be seen by the standard deviation values. The greatest variability was observed with the diet containing the lowest concentration of fiber (6.5%). Again a higher percent efficiency for iron hemoglobin repletion was observed with the diet containing 10% fiber.

Table IV. Relative Biological Values for the Experimental Diets.

DIET	RBV[1]
15 NDF 35[2]	44.33 ± 27.00
15 NDF 30	50.91 ± 17.80
15 NDF 25	56.91 ± 17.10
10 NDF 35	64.21 ± 5.00
10 NDF 30	56.49 ± 13.60
10 NDF 25	64.34 ± 11.10
6.5 NDF 35	58.64 ± 10.00
6.5 NDF 30	40.41 ± 12.50
6.5 NDF 25	57.80 ± 9.80

[1]Mean ± S.D. adjusted for body weight.

[2]15 NDF 35 = % NDF ppm iron.

Table V. Percent Efficiency of Iron Incorporation Into Hemoglobin

DIET	% EFFICIENCY
15 NDF 35[1]	15.85 ± 5.40
15 NDF 30	18.07 ± 14.10
15 NDF 25	11.70 ± 1.40
10 NDF 35	25.61 ± 6.10
10 NDF 30	22.33 ± 0.30
10 NDF 25	16.15 ± 3.70
6.5 NDF 35	20.75 ± 11.30
6.5 NDF 30	13.79 ± 2.80
6.5 NDF 25	15.77 ± 14.80
0 NDF 35	47.67 ± 8.10
0 NDF 30	43.00 ± 8.70
0 NDF 25	0.42 ± 1.10

[1]15 NDF 35 = % NDF ppm iron

It appears that a relationship may exist between iron availability and an optimum level of fiber in the diet. The ratio of corn tortillas and cooked beans was 1.78 in this diet as compared to 1.58 and 1.98 for the other test diets.

Effect of Fiber on Iron Availability

Miller in 1976 reported a 55% RBV value for raw corn and Sgarbieri et al. in 1979 reported a range in RBV values from 13.75% to 17.50% for dry beans. Values have not been reported for a combined diet of beans and corn tortillas.

Statistically there were no significant differences on iron availability due to the different fiber levels and the different corn tortilla concentrations in the test diets did not seem to effect iron availability.

The highest RBV values were obtained when the diet contained 10% fiber. The iron from a combined diet of corn tortilla and cooked beans is not readily available if one compares the RBV values obtained in this study with those published for other diets (Chao-Lo, 1979). A reduction in iron availability from the corn tortilla and cooked beans could have occurred during the cooking process. Lee and Clydesdale (1980) have suggested the processing of foods affects the chemical form of the mineral which seems to be important in iron absorption.

Effect of Total and Ionizable Iron on Iron Availability

A significant linear relationship ($P < 0.1$) was found between the total iron concentration in the diet and the percent efficiency of iron availability. This suggests that the level of iron in the diet is much

more important than other diet components in predicting the iron availability from such a combined diet. A good correlation, ($r = .43$) significant at the 5% level was obtained between soluble and ionizable iron at pH 1.35 (Table III). The ionizable iron value is lower than the soluble iron which could suggest the presence of other iron complexes which are available to the rat.

A significant correlation ($r = .46$) at the 1% level was found between the percent efficiency and the amount of soluble iron (in mg/100 g dry weight basis) as determined *in vitro*, however there was no significant correlation between the ionizable iron at pH 1.35. This may be due to low dissociation of the soluble iron complexes which will produce less free iron but at the same time some complexes from food stuffs may improve iron availability. Narasingas *et. al.* (1979) found a better correlation between the ionizable iron at pH 7.5 and the amount of iron absorbed by humans.

Difficulty in getting the experimental animal iron deficient was experienced. This was due to iron contamination in the "iron free" diet. At the onset of the experiment an iron free diet was purchased from U.S. Biochemical Corporation, Cleveland, Ohio. Initial mean hemoglobin levels were 15 g/100 ml. After receiving an iron free diet for five weeks, the rat had a mean hemoglobin level of 13.27 g/100 ml of whole blood. At this time a total iron determination of the diet showed 12 ppm of iron. A diet was then formulated in the lab as shown in Table I. Still we had approximately 12 ppm of iron in the diet. A look at the various ingredients used in the formulation showed 18.50 ppm iron in dextrin, 3.87 ppm in the vitamin mix and 42.37 ppm in cellufil. Undoubtedly the iron

contamination in the ingredients is responsible for the difficulty in getting animals used in this experiment at an iron deficient stage. Other researchers have reported animals were considered anemic when hemoglobin levels were in the range of 6 g/100 ml of whole blood (Pla and Fritz, 1971). An average hemoglobin range of 9.74 ± 1.4 to 10.67 ± 1.3 g/100 ml was obtained in this experiment and the animals were divided into groups and placed on the experimental diets at this point.

The weight gain, feed consumption data, and level of iron stored in the liver after the repletion period are shown in Table VI. A positive correlation between total iron in the diet and percent efficiency ($r = 0.94$) and the mg of iron per 100 g dry liver and % efficiency ($r = 0.91$) was observed. As can be seen from the data of weight gain/feed consumption, slightly lower feed efficiencies were observed between the experimental diets and the basal diets (casein diet).

A high percent efficiency was associated with low hemoglobin levels at the beginning of the repletion period. Also the animals tended to show a higher level of iron stored in the liver at the end of the repletion period. Hemoglobin levels during the repletion period may influence iron absorption as suggested by Amine et al. (1972). There is the possibility that as the rat approaches normal hemoglobin values the utilization of iron decreases and the animal is able to reach normal levels of blood hemoglobin even though the amount of iron available for absorption is low. Low levels of ferrous sulfate in the test diets may have been sufficient to meet the rats iron requirements. Additionally, because of the long period of attempting to get the rats anemic, the rats were not in a rapid

Table VI. Weight Gains and Hemoglobin Levels of Rats Fed Diets Containing Different Levels of Fiber and Iron (3 rats/group).

Diet	Initial Hb g/100 ml	Final Hb g/100 ml	Total Fe, mg/100g Dry liver	Feed Consumption g	Wt. gain g	wt. gain/ Feed Consumption
0 NDF 35[1]	10.31 ± 0.6	15.87 ± 0.9	47.25 ± 17.0	279.7 ± 59.2	47.7 ± 18.5	0.2 ± 0.1
0 NDF 25	10.67 ± 1.30	15.11 ± 0.6	24.60 ± 5.6	284.20 ± 20.7	62.0 ± 50.6	0.2 ± 0.2
0 NDF 0	10.48 ± 1.1	9.60 ± 2.20	23.66 ± 5.0	264.7 ± 29.8	52.3 ± 11.90	0.2 ± 0.1
15 NDF 35	9.74 ± 3.0	11.58 ± 1.60	23.46 ± 7.6	296.92 ± 42.32	62.0 ± 27.0	0.21 ± 0.05
15 NDF 30	9.93 ± 1.7	12.08 ± 1.50	22.04 ± 18.7	289.15 ± 38.61	52.0 ± 23.29	0.17 ± 0.06
15 NDF 25	9.98 ± 2.4	10.84 ± 1.60	19.69 ± 9.2	297.30 ± 18.59	47.66 ± 20.53	0.16 ± 0.07
10 NDF 35	9.74 ± 1.4	13.26 ± 1.30	33.27 ± 13.1	315.49 ± 25.07	61.0 ± 7.49	0.19 ± 0.02
10 NDF 30	9.80 ± 1.3	12.35 ± 1.0	37.01 ± 19.9	290.82 ± 45.83	58.0 ± 22.61	0.20 ± 0.05
10 NDF 25	10.50 ± 1.4	12.24 ± 1.5	31.33 ± 8.4	301.52 ± 41.10	55.0 ± 1.41	0.17 ± 0.02
6.5 NDF 35	10.34 ± 1.2	12.73 ± 1.30	31.85 ± 6.1	282.21 ± 25.72	49.0 ± 1.00	0.17 ± 0.07
6.5 NDF 30	10.07 ± 1.0	10.52 ± 1.1	30.41 ± 9.9	284.03 ± 62.85	59.5 ± 20.51	0.19 ± 0.07
6.5 NDF 25	10.22 ± 2.4	10.85 ± 5.0	24.55 ± 7.8	282.66 ± 32.65	52.0 ± 6.08	0.18 ± 0.02

[1] 0 NDF 35 = 0% NDF 35 ppm iron.

growth period, which would not reflect maximum iron requirements. These factors make it difficult to see possible effects on iron availability in fiber concentration.

SUMMARY

Different levels of fiber from corn tortillas did not significantly affect iron bioavailability in rats as measured by the iron repletion method. An optimum ratio of 1.78 of corn tortillas to beans seems to exist. The amount of iron in the diet and its form effects the efficiency of incorporation of iron into hemoglobin. Total iron, and soluble iron content of test diets correlate with percent efficiency. In vitro determination of soluble iron may be useful in predicting iron availability for different foods. The majority of iron in corn tortillas is soluble and therefore may be readily available. Beans contain a much higher level of total iron but the greatest proportion is insoluble. Because of the potential high availability of the iron in corn tortillas, as the amount was increased in the diet (as a means of increasing fiber) it may have in fact increased the amount of highly available iron.

Cooking increases the NDF levels in beans. This increase is due to the production of insoluble proteins, melanoidin type compounds, and possibly insoluble pigments present in the beans seed coat.

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