BIOAVAILABILITY OF ZINC FROM COTTONSEED

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF NUTRITION, TEXTILES

AND HUMAN DEVELOPMENT

BY

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DENTON, TEXAS

DECEMBER, 1981

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to those who supported her study:

To Dr. Elwood F. Reber, the major advisor, for his professional assistance, constant guidance, endurance and encouragement throughout this research.

To Dr. Florence Langford and Dr. Wilma Brown for their kindness, enthusiasm and for serving on the author's committee.

To Dr. David Marshall for his friendliness and aid in statistical analysis.

To her parents for their love, encouragement, untiring patience, understanding and financial support throughout the graduate study.

To the Natural Fibers and Food Protein Commission for partially funding this research.

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CHAPTER I

INTRODUCTION

Expanded utilization of glandless cottonseed as a potential plant protein for human consumption has promoted studies of nutritional value of the product. Food processing technology has been developed to assure destruction of toxic substances and to provide optimal quality of cottonseed. If cottonseed is to be considered as a possible protein source in developing countries and a protein supplement in developed countries, bioavailability of minerals, especially zinc, needs further investigation. Anderson, Gibson and Sabry (1981) stated that recent research has emphasized the essential role of zinc for normal growth, development and health in humans, and that populations whose dietary intake is restricted in animal products may not have zinc readily available to them. Davis and Reid (1979) stated that meat substitutes have become available in supermarkets and "health food" stores catering to the vegetarian market. More thought needs to be given to the possible effect of cottonseed on the zinc status of individuals.

Campen and House (1974) were of the opinion that zinc deficiency resulted in depressed plasma proteins, and that it could affect the deoxyribonucleic acid (DNA) and protein

synthesis. It has been speculated that zinc could be involved in the translation process. According to the present theory of protein synthesis, information is transferred from template DNA to messenger ribonucleic acid (m-RNA) to the polypeptide chain, eventually giving rise to the protein structure of specific conformation. This process may involve a metal ion or a zinc metalloenzyme (Pories et al., 1974).

A report compiled by Sandstead (1973) suggested that participation of the metal in the synthesis of nucleic acids and proteins probably correlated biochemically with adverse effects on growth when zinc was deficient. It was later shown that zinc deficiency in animals impairs the incorporation of labelled thymidine in DNA (Prasad and Oberleas, 1974; Kirchgessner, Roth, and Weigand, 1976). Prasad and Oberleas (1974) provided evidence that decreased activity of deoxythymidine kinase may be responsible for early reduction in DNA synthesis. These results were confirmed by Dreosti and Hurley in 1975. Earlier, Parisi and Vallee (1969) studied the role of zinc in enzyme function and discovered that some individuals with liver disease had abnormal zinc metabolism. Today, there are over 70 metalloenzymes associated with zinc (Riordan, 1976). At least one zinc metalloenzyme has been identified in each of the six enzyme

categories classified according to their function; namely: oxyreductase, transferase, hydrolase, lyase, isomerase and ligase (Goodhart and Shils, 1980). Although the biochemical role of zinc as a cofactor component is not completely understood, it is known that zinc plays an important role in the process of growth in experimental animals and in humans.

Cottonseed contains phytic acid and dietary fiber, both of which are said to reduce mineral metabolism (Oberleas and Harland, 1977; Ismail-Beigi, Faroji and Reinhold, 1977; James, Branch and Southgate, 1978). Others, however, have failed to confirm these findings (Davis, Hristic and Flett, 1976; Sandstead, Munoz and Jacob, 1978). The role of phytates in the absorption of zinc remains a controversy. Reinhold, Faradji and Ismail-Beigi (1975) stated that vegetarian diets high in phytates adversely affect calcium, iron and zinc absorption in man. Sandstorm et al. (1980) on the other hand, stated that a combination of high calcium content with phytic acid had a positive effect on zinc absorption.

Cottonseed exists as a marketable protein but its consumption by the world population is limited. The major problem is contributed by the gossypol-containing glands which can be overcome. Cottonseed can be grown in tropical areas where malnutrition and severe protein shortages exist.

Defatted cottonseed flour, when properly processed, has a protein efficiency ratio close to that of casein (Graham et al., 1969). Green et al. (1976) and Eboh (1980) found that glandless cottonseed flour, when mixed with other flours, not only increased the nutritional value of foods, but also scored high in terms of taste acceptability. Information concerning the biological value of cottonseed protein, calcium and phosphorous has been recorded, but there is no published information that indicates the bioavailability of zinc from cottonseed. There is a need to establish nutrient analysis for elements in cottonseed.

Zinc, like cadmium and lead, falls in the category of heavy metals and the possibility of zinc toxicity must be considered. Limited studies have shown that excessive amounts of zinc produce adverse effects in animals. No known safety limits have been set for humans; no toxic levels for zinc have been established. Straube, Schuster and Sinclair (1980) stated, "Most studies on the derangements of zinc metabolism have been related to the effects of zinc depletion in birds and mammals; comparatively few investigations have been concerned with the physiological changes due to excessive intake of zinc. However, it would appear that depressed growth and anemia are common expressions of zinc toxicity."

Until recently, clinical understanding of how to assess trace metal nutriture has lagged behind analytical technology, and as a result there is limited information on bioavailability of trace minerals from cottonseed. Recent technological advancements in atomic absorption spectrophotometry, x-ray florescence, proton beam analysis and neutron activation analysis enable measurement of minerals in body fluids and tissues. Beach, Gershwin and Hurley (1980) stated, "The recognition that trace elements are critical factors in human nutrition is a relatively recent concept. Because of their seemingly ubiquitous distribution in nature and micro-requirements for effective biological function, information regarding trace element nutriture has been slow in accumulating."

The objectives of this study were to:

1. assess the bioavailability of zinc from defatted glandless cottonseed flour and
2. study the effects of zinc toxicity.

CHAPTER II

BIOAVAILABILITY OF ZINC FROM COTTONSEED

Review of Literature

Cotton is grown throughout the world, primarily for a fiber yield for textiles and secondarily, for the oil content. The resulting by-product, the meal, is generally used as feedstuff (Cherry, Simmons and Tallant, 1976). Until recently, rarely was the seed of cottonplant used as a protein source for human consumption. Cottonseed flour produced from glandless variety of cottonseed is found to yield 60 to 70 percent of protein concentration (Martinez and Hopkins, 1975). Defatted cottonseed flour, when properly processed, has a protein efficiency ratio close to that of casein.

Graham et al. (1969) conducted metabolic studies to evaluate cottonseed flour as a sole protein source for convalescent malnourished children. Their study showed that glandless cottonseed flour (CFS) resulted in apparent nitrogen absorption of 80% as compared to 74% absorbed from milk protein.

Srikantia and Shagal (1968) observed poor clinical and biochemical responses when protein-energy malnourished

children were fed 6 grams of cottonseed protein per kilogram of body weight in an effort to reverse the acute manifestations of kwashiorkor. They stated, "The cottonseed preparation used in these trials contained 1% total gossypol and 0.05% free gossypol - levels considered permissible for human consumption by the WHO, FAO and UNICEF." Possible explanations for these results were : 1) a low lysine content in the flour and 2) an excessive level of gossypol may have limited protein assimilation.

Zinc is one of the essential nutrients with biochemical functions, many of which are linked with enzymes and DNA synthesis. Unlike calcium or iron, zinc does not impart significant structural integrity to the skeletal system, blood or organ tissues. Although 70 metalloenzymes are associated with zinc, the exact nature of the biochemical function is not known (Riordan, 1976). Since zinc is required for many enzymes, it is reasonable to speculate that the regulation of zinc metabolism is through 1) intake, digestion and absorption from the gastrointestinal tract; 2) formation and/or regulation of activity of zinc dependent enzymes; and 3) excretion in urine and feces.

Zinc balance refers to that state which exists when dietary zinc is in equilibrium with fecal and urinary output,

a state which is rarely observed. It is calculated by the following equation:

Zinc Balance = Zinc Intake - Zinc Output

Several factors influence the absorption of zinc from the intestine and, indirectly, the excretion of zinc in urine and feces. Thus zinc, like any other cation, is only partially absorbed by the intestine. In summation, two processes affect the zinc balance: 1) the total transfer from the intestinal lumen to plasma, and 2) the total transfer from plasma to the lumen, or endogenously excreted zinc.

Approximately 20 to 30% of dietary zinc is absorbed. Data on both the site(s) of absorption, and the absorption mechanism(s), whether active, passive or facilitated transport are meager. Cousins (1979) stated that the quantity of zinc that is transported to the serosal side of the mucosal cell and available for release into the blood stream appears to be influenced by the presence of metallothionein and other cellular ligands. Richards and Cousins (1975;1976) demonstrated that the absorption of zinc is inversely related to the concentration of intestinal metallothionein. Freeland-Graves, Ebangit and Henderickson (1980) stated that in rats with adequate zinc nutriture, zinc was found to be bound primarily to high molecular weight proteins in the intestinal mucosal cytosine that transported zinc to

the serosal surface. This transport allowed significant intestinal absorption of zinc. When the rats were fed elevated levels of dietary zinc, the intestinal zinc was found to be sequestered primarily with metallothionein. Since zinc bound to metallothionein remains in the cell and is not released to the serosal side, zinc absorption was found to be significantly diminished. Experiments conducted by Adham and Song (1980) indicated that zinc absorption in the rat is mediated by a transcellular transport process different from that which mediates copper and calcium. They suggested that calcium in high luminal concentration may depress zinc absorption by reducing the passive component of jejunal zinc absorption.

Zinc absorption is variable and is highly dependent upon a variety of factors. Among the factors that might affect zinc absorption are body size, level of zinc in the diet and the presence of other potentially interfering substances like fiber and phytic acid. Song and Adham (1978) showed that prostaglandin E_2 not only binds zinc but also facilitates its transport across the intestinal mucosa in the rat.

Evans (1980) stated that zinc is affected by levels of both dietary tryptophan and pyridoxine. Supplemental picolinic acid ameliorates the impaired zinc absorption caused

by a deficiency of either tryptophan or pyridoxine. Picolinic acid is a metabolic product of tryptophan and depends on pyridoxine for its production. Evans, Grace and Votava (1975), and Schricker and Forbes (1978) demonstrated that ligation of the pancreatic ducts markedly decreased the absorption of zinc. They were of the opinion that production of picolinic acid from tryptophan and secretion of picolinic acid into the intestinal lumen may be the rate-limiting step in the absorption of dietary zinc. Picolinic acid in the lumen coordinates with zinc to form a complex that facilitates the passage of zinc through the luminal membrane, across the absorptive cell and through the basolateral membrane of the cell. Evans and Johnson (1981) were of the opinion that the zinc dipicolinate complex facilitates the transport of zinc either because of specificity for receptor sites on the absorptive cells or because of a high association constant that facilitates transport not only of zinc but other divalent cations. They stated that vitamin B_6 deficiency causes impairment of absorption of dietary zinc as a result of decreased production of picolinic acid. Thus the production of picolinic acid is dependent on tryptophan and vitamin B_6, and other cations may compete with picolinic acid.

Zinc in the plasma is mainly bound to albumin, but other proteins such as alpha-macroglobulins also bind significant amounts of zinc (Prasad and Oberleas, 1970). In addition to protein-bound fractions, a small portion of zinc in the plasma exists as an ultrafiltrable fraction, mostly bound to amino acids, and a smaller fraction exists in ionic form.

In the past it has been shown that the activity of various zinc dependent enzymes was reduced in the testes, bones, esophagus and kidney of zinc deficient rats in comparison to their pair-fed controls (Prasad and Oberleas, 1974); Kirchgessner, Roth and Weigand, 1976: Prasad et al., 1967; Huber and Gershoff, 1970; Prasad, 1979). Prasad and Oberleas (1976) analyzed zinc from various tissues of rats. They observed that the zinc content of testes, heart, muscle, esophagus and bone decreased in zinc-deficient rats as compared to the ad lib fed controls. When the zinc depleted animals were repleted with zinc, the zinc content of testes and bone increased significantly. The activities of enzymes lactic dehydrogenase (LDH), malic dehydrogenase (MDH), antidiuretic hormone (ADH) and nicotinamide adenine dinucleotide (NADH) diaphorase were reduced in the testes of zinc deficient rats compared to the

control pairfed ad lib diets. In the repleted group the activities of these enzymes in the testes were increased.

Lei, Abbasi and Prasad (1976) investigated the role of zinc in gonadal function in rats. The increases in leutinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone were assayed following an intravenous administration of synthetic leutinizing hormone-releasing hormone (LH-RH). The figures obtained from these assays indicated that the body weight gains, zinc content of testes and their weights were significantly lower in zinc deficient rats as opposed to the control rats. The serum LH and FSH responses to LH-RH administration were higher in zinc deficient rats but the serum testosterone responses were lower compared to the restricted fed controls. These studies demonstrated a specific effect of zinc on the testes.

Franz, Kennedy and Fellers (1980) studied the bioavailability of zinc in rats using weight gains and zinc content of femurs and livers. They found that the slope ratio assay of weight gains to zinc bioavailability explained a higher proportion of true measure than similar slopes for zinc in femurs and livers versus zinc bioavailability. They concluded that there were unknown factors other than dietary zinc that determined the zinc content of femur. They supported Lei, Abbasi and Prasad (1976), stating that the

liver zinc concentration was not a sensitive indicator of the status of zinc nutrition.

Alcohol dehydrogenase found in the liver oxidizes ethanol and other primary and secondary alcohols, including vitamin A alcohol and reduces retinene. Retinene reductase of the retina is identical to this enzyme from the liver. Zinc in this enzyme is essential not only for the catalytic function but also to maintain the subunit structure (Goodhart and Shils, 1980). In another study, alcohol dehydrogenase was assayed in subcellular fractions of liver and retina from zinc deficient and control rats using retinol and ethanol as substrates (Huber and Gershoff, 1975). The activity of alcohol dehydrogenase was significantly decreased as a result of zinc deficiency in growing animals. In older rats, however, no changes in liver zinc and activity of alcohol dehydrogenase were observed. Data from these studies showed that zinc is required for the metabolism of vitamin A as well as for the catabolism of ethanol.

In several studies, the activity of alkaline phosphatase was found to be reduced in bones from zinc deficient rats, pigs, cows, chicks and turkeys (Kirchgessner, Roth and Weigand, 1976). The activity of alkaline phosphatase may be reduced due to zinc deficiency. There may not only be a loss of activity due to lack of sufficient zinc for maintaining

enzyme activity but also a diminished amount of apoenzyme due to decreased synthesis or an increased degradation (Prasad, 1979). Goodhart and Shils (1980) stated that zinc atoms of alkaline phosphatase appear to be essential for the catalytic function and subunit structure of the enzyme. They were of the opinion that zinc deficiency may also be attributed to failure of active holoenzyme.

Carboxypeptidase A and B, present in the pancreatic juice, contain zinc which is thought to be indispensible for catalytic activities that hydrolyze the peptide bonds to liberate amino acids from the carboxyterminals of proteins and peptides. These enzymes are excreted in the gastrointestinal tract and are implicated in the proteolysis and digestive process (Goodhart and Shils, 1980).

Zinc deficiency in young rats decreases DNA biosynthesis of the nuclear DNA in liver parenchyma cells. Maternal zinc deficiency impairs DNA synthesis in the rat embryo. The most convincing evidence that zinc has a specific effect on DNA synthesis comes from observations of Slater, Mildvan, and Loeb (1971), who demonstrated that DNA polymerase contains zinc. They concluded that zinc plays a specific role in both DNA and RNA synthesis, but they found no evidence to indicate that zinc is directly related to the translation process.

As earlier stated, Prasad and Oberleas (1974) were of the opinion that decreased activity of deoxythymidine kinase may be responsible for a reduction in DNA synthesis. Continuing with investigation in this area, Dreosti and Hurley (1975) found that the activity of deoxythymidine kinase was significantly lower in 12-day-old fetuses taken from female rats exposed to dietary zinc deficiency during pregnancy, as compared to those fed ad lib diets and restricted fed controls. Terhune and Sandstead (1972) and Scrulton, Wu and Goldthwait (1971), working independently, showed that livers from zinc deficient rats incorporated less phosphorous-32 into the nucleotides of RNA than did livers from pair fed controls. DNA dependent RNA polymerase was shown to be a zinc dependent enzyme.

Sandstead, Hollaway and Baum (1971) observed abnormal profiles in the livers of zinc deficient rats and mice. Administration of zinc seemed to stimulate the polysome formation. These studies suggested that zinc may have a primary effect on zinc dependent enzymes that regulate the catabolic and biosynthetic rate of RNA and DNA and play a role in the maintenance of polypeptide conformation (Prasad, 1979).

Some of the dramatic symptoms in zinc deficiency are dwarfism, retardation of sexual maturity, impairment of taste acuity and impaired wound healing (Prasad, 1979; and Sandstead, 1973). Moynahan (1976) drew comparisons in manifestations of acrodermatitis, enteropathica, i.e., apathy, alopecia, scaly skin lesions, diarrhea and those of kwashiorkor. Campen and House (1974) reported that rats fed a protein deficient diet had lower tissue concentration of zinc than did rats fed a recommended diet adequate in protein. The authors were of the opinion that when growth of rats was limited by protein deficiency, certain tissues apparently were unable to maintain zinc concentrations in the plasma and liver. Similar observations were made by Canfield et al. (1980) when treating two protein-energy malnourished children.

Beach, Gershwin and Hurley (1980) observed that dietary deprivation of zinc in developing rats produced marked and frequent physical anomalies. The rats remained hairless for five to six weeks of age, and a high rate of mortality was observed between the ages of four and eight weeks. Offspring of mice fed deficient levels of dietary zinc also exhibited extensive dermatitis as compared to animals fed diets containing zinc in adequate amounts. Progeny of zinc deficient rats demonstrated a greatly impaired rate of

growth, and the magnitude of growth retardation was closely associated with the extent of zinc deficiency.

Distinctive patterns of abnormalities found in the fetal alcohol syndrome are characterized by prenatal and postnatal growth deficiency, microcephaly, cleft palate, short palpebral fissures and joint abnormalities (Jones et al., 1973; Clarren and Smith, 1978; Hanson, Streissguth and Smith, 1978). Features observed in fetal alcohol syndrome are strikingly similar to those reported in rat fetuses when zinc intake was restricted during the crucial stage of gestation (Beach, Gershwin and Hurley, 1980). Extensive alcohol intake may deplete the body zinc store so that the role of zinc in DNA synthesis and cell division may be hampered. "It is also likely that some of the clinical features of cirrhosis of the liver such as loss of body hair, testicular hypofunction, poor appetite, mental lethargy, difficulty in wound healing and night blindness may be related to the secondary zinc deficient state in this disease" (Prasad, 1979).

Cottonseed contains phosphorous and 75% of the phosphorous is in the form of phytic acid. Phytic acid is inositol polyphosphate and forms insoluble complexes with calcium within the lumen of the intestine, thus causing a serious decrease in the absorption of available dietary

zinc (McBean and Speckman, 1974; and Wills, 1973). Sandstorm et al. (1980) observed that calcium increases precipitation of insoluble zinc phytate complexes at the intestinal pH, which is believed to be the explanation for negative effects of calcium on zinc absorption. Davis and Nightingale (1975) found that the addition of sodium phytate to yield 1% of dietary phytic acid greatly reduced rat growth and absorption of zinc. Reinhold, Faradji and Ismail-Beigi (1975) added that phytates bind minerals like zinc, calcium and iron and render them unavailable for absorption, resulting in a deficiency of these minerals in animals. Oberleas, Muhrer and O'Dell (1966) used growth rate of weanling rats to determine the effects of phytate, calcium and ethylenediaminetetraacetate (EDTA). Their study indicated that phytates decreased zinc availability and this effect was augmented by excess dietary calcium. They stated that calcium had no effect in the absence of phytates and that the effect of calcium on zinc may be mediated through an interaction of phytates. Also EDTA increased growth rate in absence of phytates. It was thought that EDTA increased zinc availability by competing with phytates and forming a soluble complex(s) which allowed absorption.

King, Stein and Doyle (1981) studied the effect of vegetarianism on the zinc status of pregnant women. The availability of zinc was questioned because of the high phytic acid and fiber content of plant foods. They found that the dietary plasma, urinary and hair zinc levels did not differ significantly between pregnant vegetarian and pregnant non-vegetarians. The plasma zinc values were about 21% lower in pregnant women as compared to non-pregnant, non-vegetarian women although the pregnant group consumed twice as much zinc as the non-pregnant women. Sandstorm et al. (1980) demonstrated that foods high in calcium, like milk and cheese, in combination with a high phytic acid content had a positive effect on zinc absorption in man. Two human metabolic studies (Reinhold, Faradji and Ismail-Beigi, 1975; and Oberleas and Harland, 1977) have shown that dietary fiber acts as a weak cation exchange resin, and binds calcium, iron and zinc, thus making these minerals unavailable for absorption. Others found that cellulose added to the daily diet of three human subjects seemed to increase the fecal excretion of zinc (Standstead, Munoz and Jacob, 1978), while Davis, Hristic and Flett (1976) maintained that phytate rather than fiber was the major determinant of zinc availability. They found that extracted bran fiber diets and zinc-

adequate control diets resulted in equal growth rates, but the growth was reduced when diets contained phytic acid and bran.

A decrease in serum zinc has been observed in human subjects consuming cellulose supplements (Tsai and Lei, 1979). The authors were of the opinion that reduced availability of zinc in the presence of cellulose may increase absorption. It was observed that cellulose, when added to a purified diet in amounts up to 16%, seemed to have no detrimental effect on the distribution of zinc in tissues. Reinhold et al. (1976) observed a decreased absorption of zinc in fiber. Reilly (1979) studied the bioavailability of zinc from diets containing soy concentrate. It was found that zinc was not readily available from soy products, especially soy concentrate. The author speculated that the differences in the zinc bioavailability were due to varying food processing conditions used in the manufacture of soy protein products. Soybean products also contained appreciable amounts of dietary fiber.

Different dietary casein concentrations were reported not to affect the mineral retention with different levels of zinc intake (Van Campen, 1973; 1972; Layrisse, Martinez-Torres and Gonzalez, 1974). Forbes and Parker (1977) demonstrated that the zinc in fullfat soy flour added to egg

white-based diets was less utilized than when zinc was added as zinc carbonate to egg white diet. Sandstorm et al. (1980) stated that zinc absorption was higher at higher protein levels. They proposed that protein or peptides formed complexes with zinc which overcame absorption-depressive agents. Another probability offered was that peptides or amino acids facilitate the absorption of zinc at the brush border level of the small intestine. Growth responses of chicks and rats were used to evaluate the biological availability of zinc in selected cereal grains and animal products, namely soybean and casein-gelatin. Observation from this study indicated that zinc from plant sources was less available than zinc from animal products (O'Dell, Burpo and Savage, 1972). Forbes and Parker (1977) found that weight gain and femur responses to increasing levels of zinc carbonate were not influenced by the presence of soy flour in the diet. Greger and Snedeker (1980) were of the opinion that the level of protein and phosphorous affect zinc absorption. Urinary zinc excretion was significantly greater when subjects consumed high protein diets rather than low protein diets. Zinc in tissues decreased when dietary phosphorous levels were increased. They concluded that the apparent zinc retention

was the greatest when subjects were fed high protein-moderate phosphorous diets.

Campen and House (1974) reported that retention of zinc by the rats was influenced significantly, both by dietary protein and by dietary zinc. The authors were of the opinion that the source of dietary protein can have marked effects on zinc metabolism, and stated that the amount of zinc that an animal can utilize efficiently would depend on the amount of protein in the diet.

Existing protein-zinc interrelationships can be viewed from two perspectives. One is to view the effect of dietary protein on zinc availability and utilization, the other is to consider the effect of zinc on protein utilization and metabolism. Some studies have shown that zinc requirements are dependent on the level and source of proteins (O'Dell, 1968; and O'Dell, Burpo and Savage, 1972). Other studies indicate that protein utilization and metabolism are reduced in zinc deficient animals (Macapinlac et al. 1968; Caldwell and Oberleas, 1969). Magee and Grainger (1979) stated that all animals receiving low zinc diets exhibit poor growth rates regardless of the level of protein. Their study was supported by Motsinger and Magee (1980) using different quality proteins such as casein, egg white solids, soy proteins and wheat gluten. The study indicated

that soy protein may be equal in quality to animal protein provided soy protein is supplemented with zinc.

Tannins and tannic acid also affect absorption of minerals from the diet. Hanny et al. (1978) stated that tannins and tannic acid present in cottonseed affected the growth of larvae of budworm tobacco. They fed developing cream or yellow anthers of cottonseed to larvae of budworm tobacco to compare the effects of cream and yellow anthers on larval growth. Growth was significantly suppressed by 15% when the larvae were fed yellow anthers compared to larvae fed with cream anthers. Gossypol-related turpenoid aldehydes (Lukefahr, Bettger and Maxwell, 1966; Bell and Stipanovic, 1977), condensed tannins (Chan and Waiss, 1978), and flavanoids (Shaver and Lukefahr, 1969) isolated from whole flower buds of cotton, are reported to inhibit growth in larvae. The effect of tannins in humans is not known, however Disler et al. (1975) and Roy (1978) reported that tannins and tannic acid clearly suppress mineral absorption when present in sufficient amounts. Tannins also preserve the seed from decay during storage. Thus, if cottonseed is to be incorporated into foods for human consumption, the fiber content, flavanoids, the gossypol-related turpenoid aldehydes, and different hybrids of cottonseed should be considered in relation to mineral availability.

Methods and Procedure

The experimental units in this study consisted of eight male, weanling, albino rats of the Holtzman strain. Initial weights of the rats varied from 61 to 67 g. The average weight of the rats was 64 g.

The rats were housed in an air-conditioned room at $22\pm1^{\circ}$ C. A twelve hour light-dark cycle was provided. Each rat was housed individually in metabolism cages to carry out the balance study. A measured amount of food was provided in metal cups specifically designed to minimize food spillage. Distilled water was supplied ad lib. All equipment used in the experiment was washed in 10% nitric acid to avoid zinc contamination.

The duration of the study was 30 days. A two day acclimation period was allowed for recovery from shipping prior to the study. During this period, the rats were fed a commercial (Purina 5001) rodent laboratory chow. Rats were fed the experimental diet containing cottonseed for a period of 28 days. During the latter half of the study two 7-day collections of urine and feces were made.

Glandless defatted cottonseed flour used in the diet was analyzed to determine the zinc content and proximate analysis (Table I). The diet containing cottonseed flour, designed

TABLE I

Proximate and zinc analyses of cottonseed flour

	Defatted cottonseed flour (%)
Moisture and Volatiles	9.0
Ash	7.43
Oil	3.24
Protein	55.41
Crude Fiber	0.80
Gossypol (free)	0.044
Nitrogen Free Extract	24.12
Zinc	0.0092[a]
	0.0081[b]

a Analysis by commercial laboratory.

b Analysis by author.

to meet the National Research Council (1978) requirement for zinc and glandless cottonseed flour, provided the required 12 mg zinc per kg diet. The diet was calculated to supply 23% protein, 5% fiber, 10% fat, 5% ash, 1% vitamin mix and 52% carbohydrate. The 5% calculated ash did not include ash from cottonseed flour. The protein content of the diet was supplied by a combination of defatted glandless cottonseed flour and spray dried egg albumin (Table II). The diet was based on the Murthy, Klevay and Petering diet (1974).

Feed intake was recorded during the balance study period. The rats were weighed each week. Weekly collections of urine and feces were made at the end of weeks 3 and 4. Samples collected were analyzed for zinc content.

Three samples of the diet containing cottonseed were analyzed for zinc. Mettler H-5 Analytical Balance was used to weigh 500 mg, 1g, 1.5g, 3g, 4g and 5g of the diet. The samples were then transferred into separate 100 ml kjeldahl digestion flasks and predigested with 10 ml of concentrated nitric acid. The samples were digested by wet ash method until the final volume was 3 to 4ml. The solutions were allowed to cool and then made up to volume with distilled water in 100 ml volumetric flasks. The diluted samples were then aspirated in Varian 475 Double Beam Atomic Absorption Spectrophotometer and analyzed at a wavelength of 213.9

TABLE II

Composition of the diet containing cottonseed flour

Ingredients	Percent
Cottonseed flour[a]	13.0[b]
Egg Albumin[c]	15.7
Cellufill[c]	4.9
Cornoil[d]	9.6
Dextrose[c]	51.8
Zinc free salt mix[e]	5.0
AIN Vitamin mix[f]	1.0

a Obtained from Rogers Delinted Cottonseed Co., Waco, Texas. Processed by the Food Protein Research and Development Center, Texas A & M University, College Station, Texas.

b Contained 11.9 g total solids.

c U.S. Biochemical Corporation, Inc.

d Mazola Corn Oil, Best Foods, CPC International, Inc.

e Zinc free salt mix no. 22683. U.S. Biochemical Corporation, Inc.

f AIN Vitamin mixture 76, (Anonymous, 1977) U.S. Biochemical Corporation, Inc.

nanometers. Zinc standard solutions were used to calibrate the instrument against distilled water blank. Absorbance readings obtained were plotted on a graph to check for linear response and to obtain zinc values. Proper dilutions were made to obtain readings on the instrument.

Urine samples were collected at the end of third and fourth week. Containers used to collect the urine samples were washed in 10% nitric acid. The actual urine volume was not determined. Possible contamination of urine samples with water, feed and fecal matter allowed for digestion with 10 ml of concentrated nitric acid. The samples were digested until the total volume was reduced to 3 to 4 ml. These samples were diluted to 100 ml, in 100 ml volumetric flasks and then analyzed for their zinc content. Urine samples were analyzed in duplicate.

Fecal samples, like the urine samples, were collected at the end of weeks 3 and 4. Total collections for each week were weighed. An analytical balance was used to weigh 1g of the fecal matter which was transferred to a 100 ml kjeldahl flask, and digested with 10 ml of concentrated nitric acid. The remainder of the procedure was the same as described for food analysis. Fecal samples were analyzed in duplicate.

At the end of the experimental period, the animals were anesthesized with ether and blood was drawn by heart puncture. Blood collected was allowed to stand at room temperature for 1 hour to allow separation of serum from the clot. The decanted serum was centrifuged for 10 minutes, decanted again and frozen until it was analyzed. The frozen serum samples were allowed to thaw at room temperature, centrifuged for 10 minutes and diluted with distilled water. The viscosity of the standard solutions was adjusted with glycerine to match the viscosity of the diluted serum (Fernandez, 1971). The samples were then aspirated in the atomic absorption spectrophotometer.

The animals were sacrificed on the last day of the experiment and the testes were removed for analysis. The testes of each animal were weighed and then digested with 5 ml of concentrated nitric acid by wet ash method. The remaining 1 to 2 ml were made to volume with distilled water in 50 ml volumetric flasks. These samples were analyzed for zinc. Statistical Package SPSS was used to analyze significant correlation data from the study.

Results and Discussion

Results from the four week study indicated a direct relationship between weight gain, feed intake and resulting zinc intake (Table III). Zinc intake was directly proportionate to the weight gains with significant correlations ($P < 0.001$; $r = 0.9627$). Legumes are good sources of plant protein, but they are considered potential reducers of mineral bioavailability because of their fiber and phytate content (Davis and Nightingale, 1975; Forbes and Parker 1977). Davis and Nightingale (1975) observed that dietary phytic acid greatly reduced rat growth and absorption of zinc. Cottonseed contains phytic acid and dietary fiber and observations from this study indicated that rats gained weight at a steady rate and thrived on the diet containing cottonseed. Proportionate weight gains were observed with increase in the zinc intake. The zinc intake was a calculated result from the feed intake. As expected, there was a significant correlation ($P = 0.001$; $r = 1.0$) between the feed intake and the zinc intake. Calculated feed intake however, did not show a strong correlation ($r = 0.5705$; $P = 0.071$) with feed efficiency. Although the feed intake varied from 234.6 to 383.5 g, the feed efficiency ranged from 0.315 to 0.398 with a mean 0.364 ± 0.027. It is possible that processing of glandless defatted cottonseed

TABLE III

Food and Zinc Intake and Effect on Growth of Rats Fed Cottonseed Diet for Four Weeks

Rat	Initial Body Weight(g)	Weight Gain (g)	Food Intake (g)	Zinc Intake (g)	Feed Efficiency
1	61	103	275.7	3.46	0.374
2	62	88	256.9	3.21	0.343
3	63	74	234.6	2.93	0.315
4	64	146	383.5	4.79	0.381
5	66	119	318.7	3.98	0.373
6	66	129	369.8	4.62	0.349
7	67	122	319.0	3.99	0.382
8	67	129	324.4	4.05	0.398
Mean	64.50	113.75	310.33	3.878	0.3644
±S.D.	2.33	23.78	52.13	0.649	0.0268

flour may have reduced the fiber and phytic acid content and that zinc present in the cottonseed is available.

Zinc in the cottonseed did not seem to affect the growth of weanling rats adversely. Rats were observed for symptoms of deficiency during the four week study but no signs of zinc deficiency were evident.

Zinc in the serum (Table IV) was correlated ($r = 0.8007$) to the weight of the testes and zinc content of the testes ($r = -0.8602$) with significance of $P < 0.01$. Surprisingly, the blood serum was not as strongly related to the zinc intake ($P = 0.045$; $r = -0.6372$). High rate of zinc turnover, regulated by homeostatic adjustments in zinc digestion to widely varying dietary zinc intake, may be a possible explanation for the correlation between blood serum and zinc intake.

Weight of the two testis expressed as a percentage of the body weight, was correlated ($r = -0.6368$) to the body weight gain and weight of the testes ($r = 0.7242$) with a significance of $P < 0.05$. There was no statistical significance ($P < .1$) between the weight of the testes and zinc intake and zinc retention ($P < 0.26$). The zinc content in the testes however was correlated ($r = 0.7014$; $P < 0.03$) to the zinc intake and the weight of the testes ($r = -0.9072$; $P = 0.001$).

TABLE IV

Content of Zinc in Serum and Testes
and Weight of Testes

Rat	Serum	Testes		
	mcg/100 ml	Weight(g)	% Body Weight	Zinc (mcg/g)
1	176	2.0075	1.22	136
2	196	2.0300	1.35	107
3	182	1.4663	1.07	148
4	184	2.0971	1.99	134
5	176	1.8786	1.01	153
6	194	1.9830	1.02	153
7	180	2.1585	1.14	135
8	186	1.3710	1.20	92
Mean	188.63	2.5420	1.223	132.38
±S.D.	13.68	1.0455	0.167	22.04

Tsai and Lei (1979) studied the effects of various levels of cellulose, copper and zinc on tissues of rats. They fed six weanling Sprague-Dawley rats a diet containing 10 mg zinc per kg, 18 mg copper per kg and 8% cellulose for a period of 9 weeks. The zinc content of serum was 146 mcg per 100 ml. The weight gain during the 9 week study averaged 263 g. Analysis of serum in this study showed a mean of 188.6 mcg per 100 ml which is comparable to the Tsai and Lei study (1979). The weight of the testes obtained at necropsy was not published, thus comparison of the testes data in this experiment to their data is not possible.

Zinc intake during the third and fourth week (Table V) varied from 0.75 to 1.38 mg zinc. Zinc intake and weight gains during the second week of the balance study were higher than comparable data during the first week. The zinc intake was significantly correlated ($P = 0.001$; $r = 0.9965$) to the body weight gain. As the rats grew there was increased consumption of food with a corresponding increase in zinc intake. Studies (Prasad and Oberleas, 1974; Slater, Mildvan and Loeb, 1971; Dreosti and Hurley, 1975; Pories et al., 1974; Prasad, 1979) have proved that zinc plays a specific role in DNA and RNA synthesis. It is believed that if zinc were deficient, rats would not gain weight as zinc deficiency interferes with DNA synthesis and thereby cell

reproduction. Thus this study indicates that zinc intake from the diet containing cottonseed flour does promote weight gain in the experimental rats.

Analysis of feces excreted by the rats for zinc (Table X, Appendix) exhibited a range of 0.47 to 0.94 mg $\pm$ 0.11. Fecal zinc during the fourth week was 70.7% of the zinc intake as compared to 62.2% fecal zinc during the third week. As seen from Table X, a direct relation existed between zinc intake and zinc excreted.

Compared to the fecal zinc excretion, an average of only 3.4% of the intake was excreted through the urine. Urinary zinc excretion values ranged from 0.020 to 0.54 mg during the third and fourth week of the balance study period. It is likely that these variations existed due to individual differences between the rats.

Weigand and Kirchgessner (1978) provided evicence that the body attempts to control zinc balance homeostatically according to the needs, by regulating the extent of intestinal absorption of dietary zinc, and the rate of fecal excretion of endogeneous zinc. Their study showed that feeding 5.6 to 141 mg zinc per kg diet, resulted in an apparent absorption of 70 and 18%, respectively. The major loss of zinc occurred through the feces. In contrast to fecal zinc excretion, the urinary output was independent

of the varying dietary zinc intake. They were of the opinion that excretion of endogenous zinc via the kidney contributed little to the homeostatic balance. Results of the present study are in agreement with Weigand and Kirchgessner study (1978). Contamination of urine with zinc by contact with feces and food spillage may present an error and result in a higher estimation of urine values for zinc.

The bioavailability of mineral is usually expressed as a percentage absorption from the diet provided that it is at a level that does not exceed the physiological requirement. Biological availability of zinc was determined from zinc intake minus zinc excretion. Zinc retention from the diet reflects the bioavailability of zinc for zinc metabolism. Zinc retention (Table XI Appendix) by the rats varied from 0.12 to 0.58 mg during week 3 and 4. Calculated estimate of zinc bioavailability showed a mean value of 30.0% for the 2 weeks with a standard deviation of 10.2%. There was a significant correlation (P 0.01; $r = 0.7850$) between zinc retained and zinc intake. Physiologically available zinc was found to be in direct correlation ($r = 0.8262$) with the body weight gain showing a significance of $P < 0.01$. During the two week balance study period, it was observed that Rat 6 consumed 1.13 mg zinc as compared to an average intake of 1.05 mg zinc consumption in week 3, however Rat 6 did not

TABLE V

Bioavailability of zinc and effect on growth of rats fed diet containing cottonseed.

Week 3

Rat	Initial Weight (g)	Weight Gain (g)	Zinc Intake (mg)	Bioavailability of Zinc (%)
1	110	28	1.04	28.9
2	119	15	0.89	20.2
3	109	14	0.75	33.8
4	150	22	1.34	33.1
5	134	18	1.06	36.2
6	152	0	1.13	49.1
7	134	25	1.05	33.6
8	131	26	1.11	30.9
Mean	129.88	18.50	1.047	33.23
±S.D.	16.38	9.07	0.172	8.06

Week 4

Rat	Initial Weight (g)	Weight Gain (g)	Zinc Intake (mg)	Bioavailability of Zinc (%)
1	138	26	1.02	17.0
2	134	16	0.84	14.8
3	123	14	0.82	21.5
4	172	38	1.38	43.3
5	152	33	1.14	31.0
6	152	43	1.32	44.0
7	159	30	1.13	13.4
8	157	39	1.24	30.1
Mean	148.38	29.86	1.110	26.85
±S.D.	15.72	10.62	0.206	12.29

gain weight. During the fourth week, it was observed that the rat gained 43.0 g as compared to 28.0 g weight gain among the other rats in the group. It is not known why this rat did not gain weight steadily like the other animals.

The study indicated that 30% of the zinc from cottonseed was physiologically available to promote growth. Prasad (1979) stated that 20 to 30 percent of the dietary zinc is absorbed. Bioavailability of zinc is dependent on a variety of factors. Motsinger and Magee (1980) indicated that the source of protein exerted a major influence on the responses of young rats. They stated that zinc supplementation to egg white solids and soy protein diet increased weight gains in rats. Forbes et al. (1979) stated that the type of phytate-protein-mineral complex formed during the processing of plant protein, rather than specific concentrations, may be responsible for the biological availability of zinc. The present study seems to support that possibility. Defatted glandless cottonseed can thus be used as a protein supplement without any adverse effects on zinc absorption.

STUDY OF ZINC TOXICITY

Review of Literature

Critical dietary and biochemical information with respect to zinc toxicity has been very limited and contradictory. Three decades ago Sadasivan (1951) reported that high levels of dietary zinc (0.5 and 1%) resulted in reduced growth, prevented normal deposition of calcium and phosphorous in the bones of young rats, and decreased activity of liver catalase. Other reports (Sadasivan, 1951; 1952) indicated that high levels of dietary zinc are associated with decreases in the retention of phosphorous which possibly decreases both calcium and phosphorous retentions in order to retain body calcium-phosphorous ratio.

Contrary to these findings, Whiting and Bezeau (1958) reported that dietary zinc increased the retention of calcium in pigs but had no influence on phosphorous retention. Stewart and Magee (1964) studied the effects of high levels of dietary zinc on growth, bone mineralization and on the metabolism of calcium, phosphorous and magnesium of young rats. They found that significant decrease in weight gain associated with zinc toxicity (0.5 to 1.0% dietary zinc) did not occur until the second week and that

it resulted in marked decreases in bone calcium and phosphorous. Bone magnesium levels were not affected by zinc toxicity. Supplements of calcium and phosphorous alleviated the adverse effects of zinc on weight gain and on deposition of calcium and phosphorous in the bone. This study suggested that zinc, calcium and phosphorous may be competing at the site of absorption.

Adham and Song's experiments (1980) showed that the transport of zinc from the mucosa to serosa was decreased by 40% in the presence of 25.0 mM $CaCl_2$. Lower calcium concentrations, however, had no effect on zinc transport. The fact that calcium reduced the passive cation movements via shunt pathway suggests that an excessive amount of one mineral may limit the absorption of the other because both ions share a common transport mechanism. On the other hand, some workers (Berry et al., 1961; Bell and Lloyd, 1963), have shown that a high calcium content in the diet had no effect on zinc metabolism.

Hoyer and Kaare (1979) studied zinc toxicity in rats wherein rats were orally injected with 1.8 mg to 58 mg zinc daily. High zinc doses resulted in death within five days. Net absorption of zinc in the remaining rats varied from 7% in the group receiving the smallest dose, to 1.8% in the group receiving the highest dose. Muktabai et al. (1980),

conducted a short-term zinc toxicity study. They fed weanling rats diets containing 0 ppm to 500 ppm zinc phosphide. Rats were found to be susceptible to zinc toxicity. Number of deaths were the greatest at dosage of 500 ppm zinc phosphide. Food intake and body weight also decreased significantly even at the lowest dosage of 50 ppm as compared to the controls. Straube, Schuster and Sinclair (1980) fed ferrets 500 to 3000 ppm zinc in diets for six months and observed severe signs of toxicity between week 1 and 2. Death occurred within two weeks time. Lesions of diffuse nephrosis, hemorrhages in the intestine and severe macrocytic hypochromic anemia were also observed. They found an increased content of zinc in the liver and kidney tissues of the animals.

Kumar in 1976 observed a high rate of fetal resorptions in pregnant rats fed 5 to 6 times higher than normal levels of dietary zinc. Other investigators have not observed this effect with excess zinc supplementation to pregnant rats (Hurley, 1968).

Bremner (1979) stated that although zinc is not generally regarded as a toxic metal, signs of copper deficiency can develop in rats fed 5000 mg of zinc per kg of the diet. Preliminary studies carried out by Campbell and Mills (1974) showed that plasma caeruloplasmin activity reduced by 40%

in rats fed 300 mg zinc per kg diet. By further increasing the zinc intake to 1000 mg per kg, there was a further decrease in the activity of this copper-containing enzyme. In addition, reduced growth rate, reduced skeletal development and decreased copper concentration in both the liver and kidney were observed. These changes are indicative of disturbances in copper metabolism induced by zinc. Solomons and Jacob (1981) studied the interaction of zinc and iron in human intestine. Oral administration of zinc was used as an index of zinc absorption. They found that iron and zinc in the ratio of 1:1 inhibited zinc absorption while iron and zinc ratios of 2:1 and 3:1 inhibited zinc intake. No effect on zinc absorption was observed when heme iron as heme chloride was ingested. Competitive interaction of zinc and iron was present with non heme iron, i.e., iron sulphate and inorganic zinc.

Weigand and Kirchgessner (1978) stated that the body is able to make homeostatic adjustments in digestion and retention of zinc for a balanced supply to the body tissues and organs to suit the specific needs. They conducted a 15 day balance study and fed 0.006 to 0.141 mg zinc per gram of the diet. They found that the apparent digestibility and retention of zinc responded to the varying dietary zinc levels. Intestinal absorption was greatly reduced to confine

zinc accretion during excessive zinc intake. The mean zinc absorption decreased with increasing zinc intake. The group receiving the largest intake of zinc showed a marked rise in the fecal zinc output above the increase in zinc intake. Urinary zinc on the other hand was largely independent of the dietary zinc intake. Urinary zinc excretions seemed to represent a major drain of endogeneous zinc from the body.

Various body tissues and body fluids have been used to assess zinc nutriture. The World Health Organization (1973) analyzed zinc nutriture in man, using plasma, serum, red blood cells, hair, urine, metalloenzymes, skin and nails for assessment. Whereas Campen and House (1974) analyzed plasma, liver, kidney and intestine for zinc.

Huber and Gershoff (1970) fed rats 1, 15 and 1550 ppm zinc. They observed that retention of zinc was directly related to the levels of zinc fed. Zinc loss was primarily in the feces but recovery of zinc was incomplete in the feces of rats fed high zinc diets due to diarrhea. High levels of zinc (1550 ppm) resulted in a significant increase of zinc in the liver, kidney, spleen, testes and heart; however, the increase in the pancreatic zinc concentrations was five times higher than in rats fed control or low zinc diets.

Chen, Vasey and Whanger (1977) investigated the accumulation of zinc in liver and kidney of rats fed various dietary levels of zinc. They found that zinc started to accumulate in rat liver when 1000 ppm or higher levels of zinc were fed in the diet. Most of the accumulated zinc was found to be present in metallothionein fractions. Increasing dietary zinc to 2000 ppm resulted in rapid accumulation in the liver and kidney. Since most zinc was found in metallothionein fractions, the authors were of the opinion that zinc was either recovered or released during protein degradation and transported by carrier protein in the blood.

Chvapil and Misiorkowski (1980) fed young and adult rats 0.5, 40 and 2000 ppm zinc. In addition, 3 mg zinc sulphate was injected parenterally every 12 hours to rats fed 2000 ppm zinc. They observed morphological and biochemical injury to the liver in young rats. High dosages of zinc inhibited the activity of lysyl oxidase in the granuloma tissue and significantly reduced the calcium content in the tissues. Adult animals did not respond to either zinc deficient diets or diets containing a high content of zinc. No changes in serum zinc were observed in adult rats.

Methods and Procedure

Eight male, weanling Holtzman albino rats were used in the study. The initial weight of rats varied from 61 to 67 g. The average weight of the rats was 64 g.

The experimental design used for the study of zinc toxicity was essentially the same as that used for rats fed cottonseed flour. The experimental diet was designed to provide 23% protein, 3.7% zinc free salt mix, 1% zinc in the form of zinc carbonate, 5% fiber, 10% fat, 1% vitamin mix and 55% carbohydrate (Table VI). Spray dried egg albumin was used as the sole source of protein. Feed intake and weights of animals were recorded weekly.

Procedures for analysis of the diet, feces, urine, testes and serum were identical to those defined for the group fed the diet containing cottonseed flour. All samples except blood serum were digested by concentrated nitric acid. Appropriate dilutions were made with distilled water before aspirating all samples so as to fit the ideal working ranges of 0.4 to 1.6 mcg zinc per ml.

TABLE VI

Composition of the diet containing one percent zinc

Ingredients	%
Egg albumin[a]	23.0
Dextrose[a]	55.0
Corn oil[b]	10.0
Cellufil[a]	5.0
Zinc free salt mix[a]	4.0
Zinc carbonate[c]	2.0
AIN Vitamin mix[d]	1.0

a U.S. Biochemical Corporation, Inc.

b Mazola Corn Oil, Best Foods CPC International, Inc.

c Fisher Scientific (65.5 Zn ÷ 125.5 $ZnCO_3$) 2.0%
$ZnCO_3$ = 1% Zn

d AIN vitamin mixture 76, (Anonymous, 1977)
U.S. Biochemical Corporation, Inc.

Results and Discussion

Results from the present study indicated that rats were susceptible to zinc toxicity. Rat 2 died at the end of week 1. Rat 9 replaced Rat 2. Diarrhea, weakness and progressive deterioration of hind limbs were observed during the study in three animals in addition to weight loss. Rat 5 died on the last day of the study. Huber and Gershoff (1970) observed diarrhea in rats fed 1550 ppm zinc. Campbell and Mills (1974) study showed that plasma caeruloplasmin activity was reduced by 40% in rats fed 300 mg zinc per kg diet. When zinc intake was increased to 1000 mg per kg diet, a further decrease in copper concentration in the liver and kidney was observed. Sadasivan (1951) observed that 0.5 to 1 percent zinc intake resulted in reduced growth, prevented deposition of calcium and phosphorous in bones of weanling rats and decreased the activity of liver catalase. The present study is in agreement with his study. Reduction in rate of growth and increasing difficulty in the use of hind limbs observed in this study, may be a result of decreased activity of liver catalase, caeruloplasmin or lysyl oxidase. Chvapil and Misiorkowski (1980) observed a decreased activity in lyslyl oxidase and decreased calcium content in all tissues.

The total zinc intake for a period of four weeks was correlated ($r = 0.7292$) to the total weight gains (Table VII) with a significance of $P = 0.031$. The total average weight gain for four weeks was only 19.0 g as compared to the weight gain of 113.8 g in the group fed the diet containing the cottonseed flour.

The weights of the testes (Table VIII) were significantly correlated to the weight gains ($P = 0.02$; $r = 0.7797$) and zinc intake ($P = 0.01$; $r = 0.7797$). Zinc in the testes expressed as micrograms per gram tissue was negatively correlated ($r = -0.6607$; $P = 0.037$) to the weight gains. Surprisingly, neither the weight of the testes nor the content of zinc in the testes was correlated with zinc retention in the body ($P=0.1$; $r = -0.1094$). The zinc content in the testes of this group however averaged to 364 mcg per g tissue as compared to 132.4 mcg per g in the group fed the diet containing cottonseed flour.

Chvapil and Misiorkowski (1980) fed 2000 ppm zinc in the diet and parenterally injected 3 mg zinc sulphate every three hours to weanling and adult rats. They observed that the serum zinc levels in young rats increased significantly in relation to the zinc content in the diet. The data in this study is in agreement with Chvapil and Misiorkowski's observations (1980). In the toxicity study the serum zinc

TABLE VII

Food and zinc intake and effect on growth of rats for four weeks

Rat	Initial Weight (g)	Weight Gain (g)	Food Intake (g)	Zinc Intake (mg)
1	61	22	214.0	2340
3	63	6	209.4	2289
4	65	8	260.6	2849
5	65	2	222.5	2433
6	66	25	266.9	2919
7	66	35	277.0	3028
8	67	35	300.7	3288
Mean	64.71	19.00	250.29	2735.14
±S.D.	2.06	13.76	35.23	383.98

TABLE VIII

Testes weight and zinc content
of testes and serum

Rat	Serum	Testes		
	mcg/100 ml	Weight(g)	% Body Weight	Zinc (mcg/g)
1	840	0.5008	0.60	194
3	980	0.4787	0.69	363
4	910	0.5061	0.69	468
5	Died[a]	0.5860	0.68	457
6	820	0.5974	0.65	485
7	780	0.7609	0.75	243
8	770	0.8831	0.86	340
9	650[b]	0.8932	0.78	269
Mean	821.43	0.6507	0.713	364.43
±S.D.	105.74	0.1712	0.081	114.33

a Rat 5 died at the end of the experimental period.

b Rat 9 was added at the end of week 1 to replace Rat 2.

average was 821 mcg per 100 ml as compared to 188.6 mcg zinc per 100 ml serum found in the rats fed a diet containing cottonseed flour. The serum zinc values were significantly correlated ($P<0.003$; $r<-0.9020$) with zinc intake. Serum zinc was also negatively correlated with zinc retained per kg body weight ($r = -0.8171$; $P = 0.01$).

The body weight loss was significantly correlated ($P>0.01$; $r>0.7803$) to the zinc intake during weeks 3 and 4 (Table IX). Zinc intake decreased by 137 mg in the fourth week as compared to the third week. The average weight loss during the fourth week was higher than the third week. It is possible that cumulative zinc toxicity reduced feed intake and thus resulted in weight loss. A wide variability existed between the rats in zinc intake. The intake of zinc varied from 384 to 1109 mg. Zinc intake was negatively correlated ($r<-0.8485$; $P<0.01$) to zinc retention. Rats 7, 8 and 9 were the only animals that did not lose weight during the study. Their zinc intake was greater than that of any other animals during weeks 3 and 4. However, the total zinc concentration per kg of body mass was considerably lower in these three rats as compared to the other rats in the group. It is possible that the weight gains resulted from a greater feed intake while the excess zinc was excreted through homeostatic adjustments regulated by the body

TABLE IX

Zinc intake and retention and effect of zinc toxicity on growth of rats

Week 3

Rat	Initial Weight (g)	Weight Gain/Loss (g)	Zinc Intake (mg)	Zinc Retained mg/kg Body Weight
1	79	- 5	645	847
3	95	0	384	664
4	95	- 7	728	782
5	94	- 7	563	666
6	97	-13	652	299
7	87	5	966	604
8	96	2	1004	627
9	109	11	1109	262
Mean	94.00	- 1.75	756.37	593.80
±S.D.	8.57	7.72	247.78	209.80

Week 4

Rat	Initial Weight (g)	Weight Gain/Loss (g)	Zinc Intake (mg)	Zinc Retained mg/kg Body Weight
1	74	9	560	545
3	95	-26	444	499
4	88	-15	555	585
5	87	-20	307	607
6	84	7	659	512
7	92	9	792	438
8	98	4	727	345
9	120	7	914	250
Mean	92.25	- 3.13	619.88	472.66
±S.D.	13.40	14.63	194.97	122.50

(Weigand and Kirchgessner, 1978). It is thought that the initial body weight and/or age may also influence the susceptibility of rats to zinc toxicity.

Zinc excretion (Table XII Appendix) in the feces showed variations of 266 to 1077 mg. There was a direct relationship between the zinc intake and fecal zinc excreted. Fecal zinc expressed as a percentage of zinc intake, averaged 91.8 and 91.3 percent for weeks 3 and 4, respectively.

Urinary zinc, however, was not influenced by the zinc intake. Urinary zinc excretion was only 0.14% of the zinc intake. However, zinc excretion in the urine of rats fed 1% zinc in the diet was 0.794 mg as compared to 0.036 mg urinary zinc excreted by the group fed the diet containing cottonseed flour.

The amount of zinc retained (Table XIII Appendix) averaged to 53.3 and 40.1 mg zinc for weeks 3 and 4, respectively. These values are not direct indicators of zinc concentration in relation to body weights. Absolute zinc retention values however, were used to express zinc retention per kg body weight.

Addition of 1% zinc in the diet resulted in growth reduction. There was an inverse relationship between the amount of zinc ingested and percentage zinc retained. It is

speculated that excessive zinc probably interfered with divalent elements at the absorption site. Also zinc may have reduced the activities of copper containing enzymes, lysyl oxidase and liver catalase, leading to interference in the enzyme activities.

Age and initial weights of the animals may have had an influence on the effects of zinc toxicity. The initial weight of rat 9, introduced at the end of week 1 of the study, was higher compared to the other animals. The effects of zinc toxicity were not as marked in rat 9 as those found in the other animals. This may be accounted for by the fact that the balance periods for rat 9 were for experimental weeks 2 and 3 as compared to week 3 and 4 for the remaining rats. There is a need for further investigation to study the influence of age and initial weight on zinc toxicity.

SUMMARY AND CONCLUSIONS

The purpose of this study was to assess the bioavailability of zinc from glandless defatted cottonseed flour and to study the effects of zinc toxicity.

Eight male weanling, albino rats of Holtzman strain were used for each study. The duration of each study was 28 days. Rats were weighed weekly. Diets were analyzed for zinc and food intake was recorded weekly. Urine and feces were analyzed during the latter half of the study. Serum and testes were analyzed at the end of the study.

To assess the bioavailability of zinc, 12 mg zinc per kg diet was provided by cottonseed flour. Thirty percent of the zinc was biologically available from cottonseed. Zinc intake was significantly correlated to the weight gains ($P = 0.001$; $r = 0.9627$). Blood serum zinc was significantly correlated with zinc intake ($P > 0.045$; $r = -0.6372$), but not with zinc retention. Weight of the testes expressed as percentages of body weights were correlated with body weight gains ($r = -0.6368$; $P = 0.045$), but not with the zinc intake. The content of zinc in the testes were correlated to the zinc intakes ($r < 0.7420$; $P > 0.018$).

One percent zinc in the diet was provided in the form of zinc carbonate to study the effects of zinc toxicity. Growth

reduction was evident in all the animals in this group. Body weight loss correlated significantly with zinc intake ($r>0.7803$; $P>0.01$). There was a direct relationship between zinc intake and fecal zinc excretion. Urinary zinc was independent of the zinc intake. Zinc retention was correlated with zinc intake ($r<-0.8485$; $P<0.01$). Neither the weight of the testes nor the content of zinc in the testes was correlated with zinc retention. Serum zinc values correlated with zinc intake ($r<-0.9020$; $P<0.003$).

It was concluded that:

1) the bioavailability of zinc was 30% when defatted glandless cottonseed flour was used as the sole source of zinc in a protein supplemented diet;

2) excessive zinc interferes with normal growth patterns. Zinc values found in the serum, testes and urine were 3, 4, and 22 times higher in the zinc toxicity study as compared to those found in the group fed the diet containing cottonseed flour. These values indicate a high level of zinc saturation in tissues. Findings from the present study suggest that upper limits for zinc be established for humans and indicate a need for further investigations on the effects of zinc toxicity.

Table X. Fecal and urinary zinc excretion from rats fed diet containing cottonseed flour.

Rat	Fecal Weight (g)	Fecal Zinc (mg)	Urinary Zinc (mg)
Week 3			
1	7.2411	0.702	0.032
2	6.0384	0.664	0.050
3	5.0265	0.467	0.032
4	9.5225	0.838	0.061
5	6.9693	0.634	0.039
6	5.6262	0.529	0.047
7	6.6024	0.640	0.054
8	7.4010	0.725	0.043
Mean	6.8030	0.6501	0.0446
±S.D.	1.3700	0.1148	0.0104

Rat	Fecal Weight (g)	Fecal Zinc (mg)	Urinary Zinc (mg)
Week 4			
1	7.7630	0.815	0.030
2	6.1056	0.690	0.026
3	5.8520	0.626	0.020
4	8.6887	0.756	0.028
5	7.8277	0.759	0.026
6	9.0560	0.706	0.028
7	6.8087	0.940	0.037
8	8.7490	0.840	0.023
Mean	7.6060	0.7665	0.0273
±S.D.	1.2320	0.0979	0.0049

Table XI. Balance study data from rats fed diet containing cottonseed flour.

Rat	Initial Body Weight (g)	Body Weight Gain (g)	Feed Intake (g)	Zinc Intake (mg)	Zinc Balance (mg)
Week 3					
1	110	28	82.5	1.04	0.30
2	119	15	71.3	0.89	0.18
3	109	14	60.1	0.75	0.26
4	150	22	107.1	1.34	0.46
5	134	18	84.6	1.06	0.39
6	152	0	90.2	1.13	0.56
7	134	25	83.3	1.05	0.35
8	131	26	88.7	1.11	0.35
Mean	129.88	18.50	83.48	1.047	0.354
±S.D.	16.38	9.07	13.77	0.173	0.116
Week 4					
1	138	26	81.2	1.02	0.17
2	134	16	66.9	0.84	0.12
3	123	14	65.6	0.82	0.18
4	172	38	110.1	1.38	0.60
5	152	33	90.7	1.14	0.35
6	152	43	106.0	1.32	0.58
7	159	30	89.8	1.13	0.15
8	157	39	98.4	1.24	0.37
Mean	148.38	29.88	88.58	1.110	0.317
±S.D.	15.72	10.63	16.57	0.206	0.192

Table XII. Fecal and urinary zinc excretion from rats fed a diet containing one percent zinc.

Rat	Fecal Weight (g)	Fecal Zinc (mg)	Urinary Zinc (mg)
Week 3			
1	8.6750	581	1.090
3	6.5191	319	1.200
4	11.3556	659	0.670
5	7.2050	504	0.830
6	10.0796	626	0.630
7	9.4802	910	0.800
8	9.3217	941	0.750
9	8.0336	1077	0.700
Mean	8.8338	702.13	0.8337
±S.D.	1.701	253.31	0.2050

Rat	Fecal Weight (g)	Fecal Zinc (mg)	Urinary Zinc (mg)
Week 4			
1	5.1345	513	1.110
3	8.2000	418	1.310
4	7.4187	512	0.800
5	6.0485	266	0.710
6	8.7450	612	0.540
7	9.0104	748	0.570
8	8.3216	691	0.630
9	7.9441	882	0.350
Mean	7.6030	580.25	0.7525
±S.D.	1.353	195.20	0.3158

Table XIII. Zinc retention and effect of zinc toxicity on growth of rats.

Rat	Initial Body Weight (g)	Body Weight Gain/Loss (g)	Feed Intake (g)	Zinc Intake (mg)	Zinc Retention (mg)
Week 3					
1	79	-5	59.0	645	62.7
3	95	0	35.1	384	63.1
4	95	-7	66.6	728	68.8
5	94	-7	51.5	563	57.9
6	97	-13	59.6	652	25.1
7	87	5	88.4	966	55.6
8	96	2	91.8	1004	61.4
9	109	11	101.4	1109	31.4
Mean	94.00	-1.75	69.18	756.38	53.25
±S.D.	8.57	7.72	22.66	247.78	15.99

Rat	Initial Body Weight (g)	Body Weight Gain/Loss (g)	Feed Intake (g)	Zinc Intake (mg)	Zinc Retention (mg)
Week 4					
1	74	9	51.2	560	45.2
3	95	-26	40.6	444	23.4
4	88	-15	50.8	555	42.7
5	87	-20	28.1	307	40.7
6	84	7	60.3	659	46.6
7	92	9	72.5	793	44.2
8	98	4	66.5	727	35.2
9	120	7	83.6	914	31.8
Mean	92.25	-3.13	56.70	619.88	40.10
±S.D.	13.40	14.63	17.83	194.97	5.58

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