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Bioavailability of Iron and Zinc Fortified Whole Wheat Flour in Rats

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Abstract.- The study aimed to compare the bioavailability of the elemental iron (EI), sodium iron ethylenediaminetetraacetate (NaFeEDTA), zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) in whole wheat flour (WWF). Eight groups of Sprague Dawley rats (n=5) were supplied fortified WWF for 30 days and one group served as a control. Percentage absorption and deposition of iron and zinc were taken as the indices of bioavailability. Significantly ($p<0.05$) higher iron and zinc absorption was observed in rats supplied with the fortified diet. Higher iron absorption was observed in diet groups fed NaFeEDTA, alone and with ZnO. Similarly, ingestion of $ZnSO_4$ manifested the highest absorption of zinc when given alone and/or with EI and NaFeEDTA. The plasma zinc level did not show the effect of feeding fortified diet, however the zinc concentration in the liver increased as a result of fortification. Further, the presence of zinc in the diet (WWF) might have an antagonistic effect on iron absorption in rats.

Key words: Whole wheat flour, fortification, iron, zinc, bioavailability.

INTRODUCTION

Iron and zinc are essential micronutrients for human growth, development and maintenance of the immune system. The diets that lead to iron deficiency anemia (IDA) tend to also provide inadequate zinc and inhibitors of iron absorption *e.g.* phytic acid, also inhibit absorption of zinc. Consequently, iron and zinc deficiencies may coexist in populations that consume diets with insufficient amounts of animal-source foods (Ranum, 2001). Including zinc with iron in fortification programmes for countries exhibiting problems of IDA seems beneficial as correction of zinc deficiency is likely to have a great impact on the health of a large population in the developing world (Prasad, 2003). Multiple fortification is a possible way of addressing deficiencies of two or more micro nutrients at the same time in a cost effective manner (Crista *et al.*, 2005).

Various chemical forms of iron and zinc have been tested as fortificants in the past to address mineral deficiency in populations particularly from developing countries. Choice of fortificants has been significantly important in relation to the type of vehicle used for fortification. Fortification of wheat flour has been regarded as a cost effective method to eliminate micronutrient deficiency, however, the presence of phytic acid in the cereals as a potent inhibitor of minerals is a significant issue and necessitates further investigation to explore its role in the fortification of cereal flours. There is a considerable doubt that EI powders currently used to fortify cereal flours are adequately absorbed. Worldwide percentage of wheat-flour fortification increased from 18% in 2004 to 27% in 2007 (Maberly *et al.*, 2008). NaFeEDTA has shown promising results in WWF as an iron fortificant (Hurrell, 2002). NaFeEDTA can be used as the fortificant to counteract the inhibitory effect on iron absorption of phytic acid present in wheat flour. The iron in NaFeEDTA binds to EDTA making it unavailable for binding with phytic acid in the flour, and is then released in the gut for absorption. There is no advantage in using EDTA in white flour or medium extraction flour used in yeast leavened bread making but there does appear to be good justification to use it in *atta* flour used to make unleavened bread (Hurrell, 1999). The reported bioavailability of sodium-iron-EDTA is 1.5 to 3

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times that of ferrous sulfate and should be added at one-half the level of ferrous sulfate (Nalubola and Nestel, 2000).

A great deal of controversy exists concerning Zn-Fe interaction. If iron and zinc are to be provided together, it is important to determine whether, and if so, how they interact biologically because they have chemically similar absorption and transport mechanisms and have been thought to compete for absorptive pathways (Sandstrom, 2001). The interaction between zinc and iron is primarily antagonistic. Extreme levels of dietary zinc impair iron metabolism directly or indirectly. The direct interaction between zinc and iron is in the intestine, both in the lumen at some intracellular location distal to the site of the regulation of iron absorption (Lonnerdal, 1996).

There is a concern that zinc co-fortification might reduce the absorption of iron from fortified flour. There did not appear to be any detectable effect when the minerals were in complex foodstuffs such as bread rolls or infant cereal (Davidsson *et al.,* 1995). Little research exists on the possible effect of zinc fortification on iron absorption (Lonnerdal, 1996). Some authors, however, suggested that zinc may reduce iron absorption in adults from an aqueous solution but not from complex foodstuffs (Crofton *et al.,* 1989).

Several organs, tissues and blood parameters have been proposed as indicators of mineral and trace element availability and absorption. Numerous studies explained deposition of minerals in tissues in relation to dietary intake, without considering the possibility of mineral interactions or mutual antagonism between elements after the absorptive phase (Larsen and Sandstrom, 1992a).

The bioavailability of dietary iron and zinc can be considerably low due to phytic acid, a potent inhibitor of iron and zinc absorption in particular from whole grain cereals and legumes and possibly other constituents of some plant foods (Hunt, 2003).

The objective of the present study was to determine the bioavailability of WWF fortified with iron and zinc, measuring % absorption and degree of retention of these minerals in plasma, the liver and the kidney, as indices of intestinal absorption and deposition in rats. NaFeEDTA, as a novel iron fortificant, was used to compare its bioavailability with conventional iron source (EI) in the presence of zinc fortificants *i.e*., $ZnSO_4$ and ZnO.

MATERIALS AND METHODS

Procurement of materials

WWF was procured from a local market to prepare fortified diets for rats, using mineral premix containing EI, NaFeEDTA, $ZnSO_4$ and ZnO. The iron fortificants were obtained from Micronutrient Initiative (MI) Ottawa, Ontario, Canada, and zinc fortificants were supplied by Fortitech Inc., New York, USA. Elemental iron reduced (96.0% Fe), used for fortification of wheat flour was USP/FCC grade, very fine particle size, produced by a hydrogen reduction or electrolytic process. It was black in color, magnetic and soluble in dilute mineral acids.

Forty five Sprague-Dawley rats were obtained from the National Institute of Health (Veterinary Division), Islamabad, Pakistan. The rats were randomly divided into nine groups comprising five rats in each group. One group served as a control and received unfortified WWF diet and eight groups were supplied fortified diets for 30 days. Prior to starting experimental diets, the rats were fed unfortified diet (*i.e*. control), for 4 weeks.

Preparation of fortified diet

The diets of the rats were prepared following the standards outlined in AIN-93 (Reeves *et al.,* 1993) with a minor modification. The following AOAC, methods were used to determine proximate composition of fortified and unfortified WWF used to prepare rats diets: drying at 105°C for 24 h for moisture (method 925.098); incineration at 550°C for ash (method 923.03); defatting in a Soxhlet apparatus with 2:1 (v/v) chloroform/methanol for lipids (method 920.39 C); and micro Kjeldahl for protein (N x 6.25) (method 960.52). Nitrogen-free extract was determined by difference. The proximate composition (%) of WWF (dry basis) showed crude protein 10.58, crude fat 2.29, crude fiber 2.69, ash 1.15 and nitrogen-free extract 82.38. The phytic acid content of the flour was found to be 0.91% (Akhtar *et al*., 2005). The fortificants were added to WWF as per treatment combination described in Table I to yield fortified flours. To

ensure proper mixing of these minerals in the flour, the fortificants were first blended with wheat flour at 1:4 (w/w) with a portion of wheat flour and this blend was added to the wheat flour. A volumetric screw type feeder was used to add premix to the flour. To achieve homogeneous flour and to ensure the level of fortification claimed, samples of fortified flours were collected during mixing at different times and aliquots were assayed for iron and zinc concentration. This process was carried out until the concentration of minerals was similar in the samples taken from various sections of the flour.

Table I.- Fortification levels (mg/kg) and treatment combinations of iron and zinc in whole wheat flour fed to male rats.

Diets	NaFeEDTA	Elemental iron	$ZnSO_4$	Zn O
D0	--	--	--	--
D1	60	--	--	--
D2	--	40	--	--
D3	--	--	30	--
D4	--	--	--	20
D5	60	--	30	
D6	40	--	--	20
D7	--	40	30	
D8	--	40	--	20

Table II.- Compositions of diets (g/kg) fed to the male rats.

Ingredients	Composition	R** AIN-93
Flour (750)	585 (Starch)	~600
Casein (85)	140 (Protein)	140
Canola oil (20)	40 (Fat)	40
Potato starch (35)	50 (Fiber)	50
Sucrose (75)	75 (Sucrose)	100
Mineral mix*(15)	15 (Min. mix)	35

* Modified without iron and zinc and adjustments made in view of Conc. of native macro and micro elements in the flour.
**R, recommendation, based on AIN-93M diet of adult rodents.

Casein, vegetable oil and potato powder were added to the WWF to meet protein, fat and fiber requirements of the rats, respectively (Table II) (Reeves *et al.,* 1993). No vitamin was added to the diet as WWF was considered to provide sufficient vitamins to meet requirements of the rats (Levrat-Verny *et al.,* 1999). Feeds were prepared with deionized distilled water in the form of pellets weighing 35-40 g each and baked in microwave oven before feeding.

Housing and maintenance of animals

The animals were housed singly in wire bottomed cages and maintained in a temperature-controlled room at 25±2ºC under 12 hours light/dark cycle. These rats were fed experimental diets for four weeks. The animals were allowed free access to feed and water.

Feed intake, mineral excretion and absorption

Iron and zinc intake of the rats was measured on the basis of the amount of feed consumed by the male rats in one week, taking into consideration the amount of water added to the fortified WWF for the preparation of feed. The fecal samples of each treatment group were collected daily for the corresponding week, packed in zip lock polythene bags, placed in the refrigerator and assayed for mineral content. Digestion of the fecal samples was carried out by the method outlined by Richard (1969) and iron and zinc concentration was measured by using atomic absorption spectrophotometery (Analyst 300, Perkin-Elmer, Boston, MA) by following the methods described in AOAC (2000). The mean values for the three fecal samples were taken as the amount of mineral present in the feces. Percentage absorption of the iron and zinc was calculated as

$$\% \text{ absorption} = \frac{\text{Total iron consumed in the feed} - \text{faecal iron excretion}}{\text{Total iron in the feed}} \times 100$$

Determination of mineral deposition in rats

Rats were anesthetized with sodium pentobarbital in the morning after one month of feeding fortified diets. These animals were sacrificed one by one and aortic blood was taken in labeled test tubes to separate plasma at 10,000 x g, for 5-6 minutes (Model TJ-6 with TJ-R refrigeration unit, Beckman Instruments, Fullerton, CA). These plasma samples were transferred in 1 ml centrifuge tubes and stored at -20ºC for analysis of trace elements. After blood sampling, rats were dissected and the body parts *i.e.,* liver and kidney, were removed from the carcass and were frozen before

further analyses. Digestion of the samples was carried out as described by the method of Richard (1969). 0.5 g sample of the flour was digested in 100 ml conical flask adding 10 ml HNO_3 at a temperature of 60-70°C for 20 minutes and then digested with 5 ml $HClO_4$ at a temperature of 60-70°C for 20 minutes and subsequently temperature was raised to 195°C till the sample was transparent and reduced to 1-2 ml. The digested samples were diluted in a volumetric flask with deionized water. These samples were then loaded to Atomic Absorption Spectrophotometer and concentration of iron and zinc was measured by atomic absorption spectrophotometery as described above (AOAC, 2000).

Statistical analysis

Analysis of variance was used to compare means of each form of iron and zinc in all treatment (Steel *et al.,* 1997). Duncan's Multiple Range Test was applied to assess the difference between means (Duncan, 1955). The data represent the mean of five values i.e. no. of rats in each treatment group. Statistical significance was set at $P \leq 0.05$ probability levels.

RESULTS AND DISCUSSION

Effect of zinc on iron absorption in rats

Iron intake of the male rats varied significantly ($P \leq 0.05$) with changing type of iron in the diet. The highest iron content was consumed by the rats from the diets containing EI (D2, D7 and D8) followed by the diets supplemented with NaFeEDTA (D1, D5 and D6). Diets without added iron (D0), diet with $ZnSO_4$ alone (D3) and ZnO alone (D4) manifested the lowest iron intake (Table III). The apparent absorption of iron in these rats seemed to be correlating with the iron concentration of the individual iron source supplemented in the diet *i.e.* EI and NaFeEDTA. The concentration of iron in EI was ~ 97% as compared to NaFeEDTA containing ~ 13 % iron.

The highest iron excretion through faeces was observed in the rats that consumed EI (D2, D7 and D8) and iron intake and excretion was shown to be consistent in these rats (Table III). Highest apparent absorption of iron was observed in rats groups that consumed EI with $ZnSO_4$ (D7) followed by the rats groups fed EI with ZnO (D8) (Table III). NaFeEDTA supplemented alone and with ZnO (D1 and D6) in the diets has shown the highest absorption (%) of iron in rats, however, absorption (%) of NaFeEDTA did not differ significantly ($p<0.05$) from the control when supplied with $ZnSO_4$ (D5) in the diet (Fig. 1). EI given with either source of zinc (D7 and D8) in the diets indicated a non-significant difference in iron absorption (%) as compared to the control (Fig. 1). Indigenous forms of iron in the presence of added zinc sources (D3 and D4) also showed no difference ($p<0.05$) in absorption (%) of iron in the rats (Fig. 2). Davidsson *et al.* (2002) reported better iron absorption from NaFeEDTA than from other compounds, substantiating the finding of iron absorption in the present study.

Table III.- Apparent absorption (ug /day) of iron in male rats fed fortified whole wheat flour.

Diet	Iron intake	Iron excretion	Absorption
D0	1185.38±22.72^{e}	665.75±12.81^{d}	519.50±10.31^{e}
D1	1399.50±19.71^{c}	621.88±17.16^{e}	777.63±16.27^{c}
D2	1996.38±26.18^{b}	1207.50±26.88^{b}	778.88±17.13^{bc}
D3	1177.88±21.39^{e}	689.00±14.71^{cd}	488.88±9.82^{f}
D4	1171.75±25.72^{e}	707.63±18.12^{c}	464.13±8.19^{g}
D5	1391.75±28.42^{c}	688.50±17.07^{cd}	703.25±18.33^{d}
D6	1334.38±32.57^{d}	623.13±11.09^{e}	711.25±16.83^{d}
D7	2058.13±40.58^{a}	1206.25±27.90^{b}	851.88±18.09^{a}
D8	2071.88±42.91^{a}	1277.88±29.47^{a}	797.75±21.42^{b}

Means (±SE), carrying similar alphabets in columns do not differ significantly ($p<0.05$). n=No of rats per dietary group D0=Control, D1=NaFeEDTA, D2=Elemental iron, D3= $ZnSO_4$,D4=ZnO,D5= NaFeEDTA+ $ZnSO_4$, D6= NaFeEDTA+ ZnO, D7= Elemental iron+ $ZnSO_4$, D8= Elemental iron+ ZnO. Values are the mean ± SD for five rats (n=5) at ($p<0.05$).

Type of zinc source predominantly acted in a different manner with NaFeEDTA in the diet. $ZnSO_4$ was found to have greatly reduced the iron absorption in NaFeEDTA group indicating a high level of antagonistic effect on iron absorption. Conversely, ZnO revealed no such effect on iron absorption when supplied with NaFeEDTA.

Sufficient literature is available to support a negative effect of zinc on iron absorption when supplied in the diet together. Trace element interactions are generally antagonistic and when two

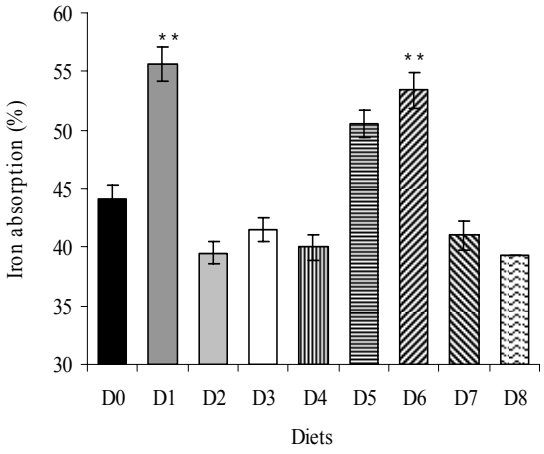

Fig. 1. Iron absorption (%) in male rats fed zinc and iron fortified whole wheat flour for one month. D0=Control, D1=NaFeEDTA, D2=Elemental iron, D3=$ZnSO_4$, D4=ZnO, D5= NAFeEDTA+ $ZnSO_4$, D6=NAFeEDTA+ ZnO, D7=Elemental iron+$ZnSO_4$, D8=Elemental iron+ ZnO. Values are the mean ± SD for five rats (n=5) at ($p<0.05$). **Indicates the values significantly different from control. D represents the type of the diet.

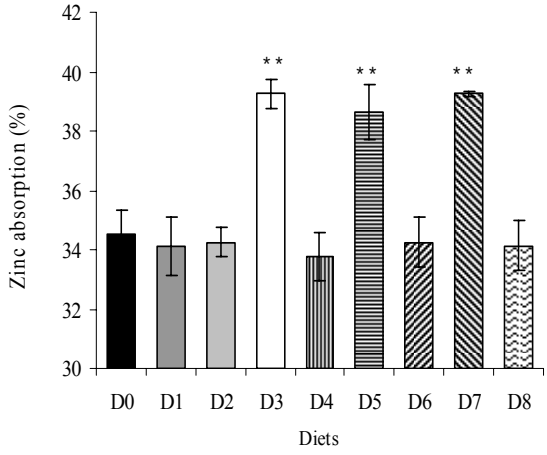

Fig. 2. Zinc absorption (%) in male rats fed zinc and iron fortified whole wheat flour for one month. D0=Control, D1=NaFeEDTA, D2=Elemental iron, D3=$ZnSO_4$, D4=ZnO, D5= NAFeEDTA+ $ZnSO_4$, D6=NAFeEDTA+ ZnO, D7=Elemental iron+$ZnSO_4$, D8=Elemental iron+ ZnO. Values are the mean ± SD for five rats (n=5) at ($p<0.05$). **Indicates the values significantly different from control. D represents the type of the diet.

chemically similar ions are present in the intestinal lumen, the one having the greater ratio tends to exclude the other (Solomons and Ruz, 1997). Another study confirmed that the ingestion of excess amounts of zinc has been found to induce anemia and depress tissue iron levels in rats and chicks (Bafundok *et al.,* 1984) but this interaction has received little attention in humans. Moreover, the basis of this interaction is not known (Valberg *et al.,* 1984). The negative effect of zinc on iron absorption was corroborated in another study (Siewicki *et al.,* 1986) where the authors confirmed inhibition of iron absorption by ingestion of excess zinc in rodents. $ZnSO_4$ and ZnO vary in their solubility ranging from very soluble to almost insoluble (Henderson *et al.,* 1995). Iron in NaFeEDTA is chelated with EDTA and in the human gut, the iron is released from EDTA allowing it to be absorbed (Hurrell, 1999). This absorption of iron from NaFeEDTA at this stage is presumably influenced by the type of zinc compound and $ZnSO_4$ being more soluble might have suppressed the iron absorption as compared to insoluble ZnO. Another study reported that iron and zinc appear to be highly bioavailable from foods made of fortified flour but $ZnSO_4$ co-fortification may have a detrimental effect on iron absorption. Iron absorption from flours fortified with iron only was good (15.9±6.8%) but when corrections were made for haemoglobin concentrations, it was significantly lower from the flour co-fortified with $ZnSO_4$ (11.5±4.9 % $p<0.05$) but not from the flour co-fortified with ZnO (14.0±8.9%) (Henderson *et al.,* 1995).

Effect of iron on zinc absorption in rats

A systematic pattern for zinc intake, excretion and apparent absorption could not be observed among various diet groups (Table IV). The highest zinc intake and excretion was observed in rats fed diets containing EI with ZnO (D8). Similarly, the highest apparent absorption of zinc was observed in $ZnSO_4$ alone (D3) and with EI (D7) (Table IV).

Data presented in Figure 2 revealed the highest ($p<0.05$) absorption (%) of zinc in the diets of male rats containing $ZnSO_4$ alone (D3) and with either source of iron (D5 and D7). Absorption of zinc from ZnO in all types of feeds with or without

iron fortificants (D4, D6 and D8) did not significantly ($p<0.05$) differ from the control diet group (Fig. 2). Conclusions that might be drawn from the existing data are that the $ZnSO_4$ is relatively more bioavailable in WWF as compared to ZnO, and the iron fortificant primarily do not have a negative effect on the absorption of zinc in the diet of the rats.

Table IV.- Apparent absorption (ug/day) of zinc in male rats fed fortified whole wheat flour.

Diet	Zinc intake	Zinc excretion	Absorption
D0	646.75±11.72 ^{f}	424.13±8.12 ^{f}	222.63±4.72 ^{ef}
D1	666.50±12.09 ^{e}	439.25±9.01 ^{e}	227.25±4.81 ^{e}
D2	637.38±12.91 ^{f}	420.63±9.12 ^{f}	216.75±4.68 ^{f}
D3	878.13±14.88 ^{d}	530.13±11.80 ^{d}	348.00±6.18 ^{ab}
D4	958.88±17.85 ^{b}	634.38±13.16 ^{b}	324.50±6.13 ^{d}
D5	905.75±18.09 ^{c}	563.50±12.72 ^{c}	342.25±5.92 ^{bc}
D6	953.00±20.08 ^{b}	626.00±13.39 ^{b}	327.00±5.81 ^{d}
D7	897.75±19.25 ^{c}	543.75±12.14 ^{d}	354.00±6.37 ^{a}
D8	980.50±20.28 ^{a}	682.88±13.91 ^{a}	338.80±6.08 ^{c}

Means (±SE) carrying similar alphabets in columns do not differ significantly($p<0.05$) D0=Control, D1=NaFeEDTA, D2=Elemental iron, D3= ZnSO4, D4=ZnO,D5= NaFeEDTA+ ZnSO4, D6= NaFeEDTA+ ZnO, D7= Elemental iron+ ZnSO4, D8= Elemental iron+ ZnO. Values are the mean ± SE for five rats (n=5) at ($p<0.05$). n=No. of rats per dietary group.

Extensive review of the literature pertaining to bioavailability of zinc from different zinc sources in various food systems and the effect of iron on zinc bioavailability revealed contradictory results. A previous study demonstrated low bioavailability of zinc from ZnO with regard to other dietary sources and was prone to antagonistic reactions with other nutrients (Darnton Hill *et al.,* 1999). However, several other researchers confirmed that ZnO and $ZnSO_4$ are equally well absorbed when added as fortificants (Davidsson *et al.,* 1995 ; Herman *et al.,* 2002).

No negative overall effect of NaFeEDTA consumption on the metabolism of zinc and calcium was observed through experimentation (Davidsson *et al.,* 1994). However, iron might have a negative effect on zinc absorption as long as given in aqueous solutions while no effect is observed when zinc is added to meals (Rossander-Hulten *et al.,* 1991). The possibility that EDTA could enhance zinc absorption in other diet types requires further investigation (Hotz *et al.,* 2005).

According to some studies, if the iron-zinc interaction is a direct result of competition for common binding sites then in addition to the shared pathway, there is also a specific absorptive pathway for iron. It therefore follows that if iron absorption via the shared pathway is compromised in the presence of zinc, then compensation occurs in terms of an increased uptake via the pathway dedicated to iron. However, it appears that this is not the case for zinc, which means that either there is no unique absorptive pathway for zinc or that, in the face of competition, iron receives preferential treatment over zinc (Susan and Susan, 1989).

Table V.- Iron content (μg/g*) in organs of the male rats fed mineral supplemented diets.

Diet	Plasma	Liver	Kidney
D0	2.82±0.17	78.97 ±4.20^{bc}	22.50 ±0.87^{c}
D1	2.96±0.20	98.03 ±7.61^{a}	28.98 ±1.62^{ab}
D2	3.27±0.20	93.50 ±6.46^{ac}	30.78 ±1.47^{a}
D3	2.68±0.09	77.71 ±3.39^{c}	26.67±1.12^{c}
D4	2.90±0.12	78.49 ±4.92^{bc}	25.08 ±1.76B^{c}
D5	3.06±0.12	91.94 ±3.74^{ac}	20.06 ±1.89^{ac}
D6	3.11±0.12	91.36 ±5.28^{ac}	28.58 ±1.24^{ab}
D7	3.06±0.11	96.50 ±4.91^{a}	30.57 ±1.74^{a}
D8	3.14±0.11	95.52 ±3.81^{ab}	28.86 ±1.90^{ab}

Means (±SE) carrying similar alphabets in the columns do not differ significantly ($p<0.05$). D1=NaFeEDTA, D2=Elemental iron, D3= $ZnSO_4$, D4=ZnO, D5= NaFeEDTA+ $ZnSO_4$, D6= NaFeEDTA+ ZnO, D7= Elemental iron+ $ZnSO_4$, D8= Elemental iron+ ZnO. Values are the mean ± SE for five rats (n=5) at ($p<0.05$). n=No of rats per dietary group *Wet Weight

Mineral retention in body organs of rats

Iron and zinc in plasma

Plasma iron and zinc concentration did not change ($p<0.05$) as a result of feeding iron and zinc fortified diets to rats (Tables V, VI). The present study demonstrated a higher concentration of iron absorption from NaFeEDTA diet groups (D1, D5 and D6) and zinc from $ZnSO_4$ diets groups (D3, D5 and D7) (Figs. 1, 2), however, the concentration of iron and zinc absorbed did not apparently raise plasma zinc concentration in the male rats. Previous studies on this subject substantiated the findings of the present results, indicating that plasma iron and zinc might not be an indicator of the mineral absorption in rats. There are limitations to the

studies on effect of iron on zinc absorption because measurement of circulating concentrations did not necessarily indicate the net zinc uptake (Sandstrom, 2001; Whittaker, 1998).

Iron and zinc in liver

Liver iron concentration of rats, fed diets containing NaFeEDTA alone (D1) and EI with $ZnSO_4$ (D5) was significantly higher than all other types of fortified diets (D2, D3, D4, D6, D7, D8) including control (D0) (Table V). However, ingestion of other fortified diets (D6, D7, D8) still indicated higher concentration of iron in the liver of male rats as compared to control (D0) and diets fortified with zinc alone (D3, D4) though the differences were non significant (Table V). The highest zinc concentration in liver was observed in rats fed diets containing $ZnSO_4$ alone (D3) followed by ZnO alone (D4) and $ZnSO_4$ with EI (D7), albeit $ZnSO_4$ with NaFeEDTA (D5) indicated a reduced liver zinc concentration in these rats (Table VI). Significantly higher zinc concentration in the liver was observed in rats fed a zinc fortified diets as compared to rats fed diets without added zinc.

Table VI.- Zinc content (µg/g*) in organs of the male rats fed mineral supplemented diets.

Diet	Plasma	Liver	Kidney
D0	1.59±0.07	30.90 ±1.17^{de}	16.17 ±1.13^{cd}
D1	1.64±0.12	31.69 ±1.83^{de}	15.03 ±0.98^{d}
D2	1.60±0.10	30.07 ±1.76^{e}	15.18 ±0.98^{ab}
D3	1.83±0.08	44.14 ±0.94^{a}	22.39 ±0.90^{ab}
D4	1.84±0.08	41.37 ±1.67^{ab}	21.53 ±0.86^{ab}
D5	1.76±0.11	35.71 ±1.64^{cd}	19.27 ±1.06^{d}
D6	1.75±0.10	36.90 ±1.85^{bc}	18.58 ±0.75^{a}
D7	1.78±0.10	41.65 ±2.07^{ab}	21.15 ±1.12^{b}
D8	1.69±0.11	38.00 ±1.43^{bc}	19.41 ±0.72^{bc}

Means (±SE) carrying similar alphabets in the columns do not differ significantly ($p<0.05$). D1=NaFeEDTA, D2=Elemental iron, D3= $ZnSO_4$, D4=ZnO, D5= NaFeEDTA+ $ZnSO_4$, D6= NaFeEDTA+ ZnO, D7= Elemental iron+ Zn SO_4, D8= Elemental iron+ ZnO. Values are the mean± SE for five rats (n=5) at ($p<0.05$). n=No of rats per dietary group *Wet weight.

The data for iron and zinc concentration of liver in male rats after feeding fortified diets corresponded to the results obtained in iron and zinc absorption in the present study (Figs. 1, 2). Reduced absorption of iron and zinc and degree of deposition of iron from NaFeEDTA and zinc from $ZnSO_4$ in the presence of one with the other have clearly confirmed the level of bioavailability of different fortificants and the antagonistic effect of zinc on iron absorption.

Previous reports have led to a variety of results concerning the interactive effect of dietary minerals on the iron content of organs and tissues. NaFeEDTA is thought to be highly bioavailable as iron from it is chelated with EDTA, a commonly used food additive which prevents the iron from being bound with phytic acid. NaFeEDTA is better absorbed and not sensitive to many food iron inhibitors if compared with other iron fortificants (Hurrell, 2002; Viteri *et al.,* 1995). Deposition of iron in organs and tissues was mainly correlated (inversely) to the zinc absorption. The absorption of zinc from dietary sources was directly proportional to the amount of zinc in the diet which was clearly indicated in the elevated zinc concentration of the liver and kidney, however, these levels of zinc from the diet decreased the iron content in these organs (Kang *et al.,* 1977; Larsen and Sandstrom, 1992b).

Iron and zinc in the kidney

The concentration of iron in the kidney of the male rats correlated with iron concentrations in the liver of these rats (Table V). Transition from one iron source to other or switching to different iron combinations with zinc sources did not show any difference in iron deposition patterns in the kidneys of these rats (Table V). Interestingly, identical results with zinc liver deposition were observed for the zinc concentration of kidneys of these rats and interaction of NaFeEDTA with $ZnSO_4$ was found to be consistent throughout the study (Table VI).

Concentration of zinc in the kidneys of the rats did not indicate any significant difference ($p<0.05$) when $ZnSO_4$ and ZnO were given alone (D3, D4) or with EI (D7, D8), however, NaFeEDTA exhibited an antagonistic effect when supplemented with these zinc sources (D5, D6) (Table VI). This has clearly shown that NaFeEDTA being highly soluble and more reactive as compared to EI has antagonistically interacted with $ZnSO_4$ and ZnO. This was supported in a study reporting that the lower solubility of ZnO might be expected to reduce

zinc absorption compared with $ZnSO_4$. The lower bioavailability of EI as compared to NaFeEDTA has been confirmed in many studies (Fritz, 1976).

In another study comparing the absorption, authors found no significant difference in zinc absorption from the two zinc salts. An alternative possibility is, therefore, that ZnO dissolves more slowly in the gut and so has higher lumen concentrations in the proximal small intestine, where the interaction with iron absorption might occur, but ultimately dissolves more in the more distal parts of the gut, allowing zinc absorption to be similar in the Fe+ZnO and Fe+$ZnSO_4$ groups. Iron and zinc were well absorbed; however, the addition of $ZnSO_4$ to the dumplings significantly decreased the amount of iron absorbed. No similar effect was seen for dumplings co-fortified with ZnO. The results suggested that caution should be exercised when considering whether to fortify iron-fortified flour, or foods made from iron fortified flour, with zinc, especially with $ZnSO_4$ (Herman *et al.,* 2002).

Zinc may reduce the beneficial effect of iron status, but this negative interaction does not appear to be great enough to discourage joint supplementation. Even in the presence of zinc, the benefit of iron supplementation on iron indicators was significant and important. Iron does not appear to have a negative effect on serum zinc concentrations; if there is an effect, it is small (Crista *et al.,* 2005).

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