INVIRO STUDY OF SOME FACTORS AFFECTING ZINC BIOAVAILABILITY

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Abstract

Five subjected groups of 5-weeks-old Swiss Webster out bred male mice were caged individually in stainless steel cages and maintained at 22-24° C and 45-55% relative humidity. The analysis of the previously prepared 5- different diets were done after complete homogenization of their ingredients. Mice group (A) were fed zinc deficiency diet *adlibitum* of "9.6 mg Zn / kg diet, mice group (B) were fed zinc controlled *adlibitum* diet "31.3 mg Zn / kg diet", mice group (C) were fed zinc supplemented *adlibitum* diet "60mg Zn /kg diet", zinc source of last groups is $ZnSO_4.7H_2O$. On the other hand, mice groups (D) and (E) were fed zinc supplemented *adlibitum* diet included phytic acid in concentration of 1497.0 and 1505.4 mg / kg diet respectively, as well as, their zinc sources are $ZnSO_4.7H_2O$ in concentration of 58 mg / kg diet in diet of mice group (D) and $ZnNa_2EDTA$ in concentration of "58.4 mg / kg diet" in diet of mice group (E). Experiments were initially designed to examine the simultaneous interaction between both zinc and other nutrient metals as calcium, iron and copper and the effect of dietary fiber of phytic acid on zinc absorption and study the impact of zinc absorption enhancement by using a chelating source of zinc as EDTA on the utilization of these nutrients. The duration of the experiment was 4- weeks divided into 2- intervals, 2- weeks for each interval. Serum calcium and iron were not affected by neither zinc deficiency nor zinc supplementation. Due to the aggressive competition between zinc and copper absorptions on the same cites of small intestine, copper absorption was positively highly affected by zinc deficient diet and recorded highly increasing in HDL- C "good cholesterol" and was negatively highly affected by zinc supplementation as hydrated zinc sulfate "$ZnSO_4$.$7H_2O$" recorded high value of LDL- C "bad cholesterol". Zinc supplementation as "$ZnNa_2EDTA$" recorded highly increasing in absorption levels of zinc and copper in blood. To the degree that affected the functions of kidney {urea and creatinine}. The majority of liver cells completely lost their cytoplasmic structure in mice of zinc deficient diet, either due to low intake of zinc or presence of dietary fiber of phytate. Phytate is known to form a complex with nutritional metals" Ca, Fe, Zn and Cu". Thus, the unavailable phytate and metallic nutrients complexed with it can not be utilized

and were excreted. Hair analysis for minerals, gives a good and sensitive indication of actual metal levels while blood levels may still be in the normal range. Fortification the floor of the balady bread with zinc in EDTA form is a good intervention for the governorates which their villages suffere from signs and symptoms of zinc deficiency.

KEY WORDS:

.Male mice	.Zinc deficiency	.Zinc supplementation	. *Adlibitum*
.Calcium	.Iron	.Copper	.Phytate
.Food consumption		.$ZnSO_4 .7H_2O$	.$ZnNa_2EDTA$
.Blood serum	.HDL- C "good cholesterol"		.LDL- C "bad cholesterol"
.Alkaline phosphatase		.Urea	.Creatinine
.Histopathlogical studies		.Lesions	.Hair analysis

INTRODUCTION

Zinc deficiency in human is widespread throughout the world, it is more prevalent in areas where the population subsists on cereal proteins. Zinc deficiency is seen in many disease states, its deficiency during growth periods results in growth failure and lack of gonadal development in males Prasad, (1995). After iron, zinc is the second most abundant trace metal in the human body where, an average 70-kg adult human contains 2.3g of zinc. Zinc as an essential mineral in animal diets, it has been demonstrated for the growth, development and differentiation of all living organisms, including microorganisms, plants and animals. More than 300 zinc enzymes covering all six classes of enzymes in different species of all phyla have been discovered. Zinc can be measured in blood "plasma or serum", feces, urine, saliva, and hair. Many Zn-salts are highly soluble in water, zinc and its salts are highly persistent in water with half lives >200 days Keith *et al.* (2000). Zinc deficiency may arise from low dietary intakes, low bio availability and/or interactions with other nutrients and losses through disease processes e.g. diarrhea Ronette *et al.* (2000). The discovery of zinc deficiency in humans occurred in populations characterized by diets of reduced bio-availability of metallic elements due to high concentration of phytate and low protein content Bolonnerdal, (2000). Signs and symptoms of dietary zinc deficiency include, growth retardation [30% of the world's children are stunted as a result of zinc deficiency], sexual immaturity [inadequate sexual development in children and adolescents], skin change" an acne-like rash", hair loss [hair and nail growth only consume 0.5 µmol/day], immunologic abnormalities and loss of appetite [Severe Zinc Deficiency], Mental confusion, impaired wound-healing, reduce senses of taste and smell, Walsh *et al.* (1995), Prasad, (1998) and Ronette *et al.* (2000). Zinc deficiency also affects fetal growth. Zinc supplementation during pregnancy reduce risk of diseases [diarrhea or impetigo] in small-for-gestational-age but not preterm infants,

however, early zinc supplementation in low birth weight or small-for-gestational-age infants reveals an effective improvement in growth, which suggests a prenatal depletion Carlos and Gerardo, (2003). Low zinc intake in children decreases the psycho educational performance, poor maternal zinc status in pregnancy can have adverse effects on fetal brain function and low birth-weight infants Michael, (2000). The role of zinc in children's cognitive and motor functioning is usually assessed by the response to supplementation in populations thought to be zinc deficient Maureen, (2003). Recently, Alberto *et al.* (2003) reported that zinc deficiency induced ulcerations, inflammation and mucosal damage in rat intestine through out the experimental period.

The purpose of this research was to study the simultaneous interaction between both zinc and other nutrient metals as calcium, iron and copper and the effect of dietary fibers on zinc absorption and study the impact of zinc absorption enhancement by using a chelating source of zinc as EDTA on the utilization of these nutrients.

MATERIALS AND METHODS

Animals 5-weeks-old Swiss Webster out bred male mice were divided into 5- groups, caged individually in stainless steel cages and maintained at 22-24° C and 45-55% relative humidity. Acid-washed glass food jars and polyethylene bottles with polyethylene stoppers were used. Diets and de-ionized water were provided fresh daily *adlibitum* unless otherwise specified. All the utensils used in providing the diets were either stainless steel or acid washed glass Stahr, (1977).

Diets five diets were previously prepared according to the composition of basal diet which is shown in table 1 Pang *et al.* (1992) and NRC, (1995) for the 5- subjected experimental animal groups. The 5- diets were different not only in amount and source of zinc but also in nature of dietary fiber ingredients of the composition of basal diet.

Table 1. Composition of basal diet:

Ingredients	Concentration (%)
Casein	20.0
DL-methionine	00.3
Sucrose	50.0
Cornstarch	15.0
Fiber* (cellulose or wheat bran)	05.0
Corn oil	05.0
Mineral mixture	03.5
Vitamin mixture	01.0
Choline bitartrate	00.2

*The dietary fibers of *adlibitum* diets of animal groups (A), (B) and (C) are cellulose while in groups (D) and (E) are wheat bran.

Experimental designs experiments were initially designed to examine the simultaneous interactions between zinc and other nutrient metals as calcium, iron and copper and the effect of phytic acid of dietary fiber on zinc absorption and study the enhancement of this absorption by using a chelating source of zinc as EDTA. The duration of these experiments was 4- weeks divided into 2- intervals, 2- weeks for each. Mice in these experiments were housed individually in stainless steel cages as shown in fig 1 were previously randomly distributed on 5- subjected experimental groups and were randomly assigned to one of the following experimental groups of 14 mice each. Zinc deficiency mice group (A) were fed the zinc free diet *adlibitum*, control mice group (B) were fed zinc control diet *adlibitum* "30 mg Zn / k diet as $ZnSO_4.7H_2O$", zinc supplemented mice group (C) "$ZnSO_4.7H_2O$" were fed zinc supplemented diet *adlibitum*, "60 mg Zn / k diet as $ZnSO_4.7H_2O$", zinc supplemented mice group (D) as "60 mg Zn as "$ZnSO_4.7H_2O$" / k diet *adlibitum* " in presence of wheat bran fiber as natural source of phytic acid and finally, zinc supplemented mice group (E) were fed zinc supplemented diet *adlibitum* as "60 mg Zn /k diet as $ZnNa_2EDTA$" in presence of wheat bran fiber as natural source of phytic acid. All other nutrients e.g. Ca, Fe and Cu were added according to the recommended dietary allowance [RDA] for each of the mice and according to NRC, (1995). All groups were eaten barley for 3- days before starting the experimental period in purpose of to be adapted. Two animals from each group were randomly withdrawn, representing zero time samples for all groups, then, each group contained 12- male mice. After 2- weeks, six mice were scarified from each group and considered the first interval. According to Waynforth and Flecknell, (1992) mice of both zero time and each interval were anaesthetized with ether, hair samples were taken as well as blood samples were collected by cutting the armpit vein. Then, mice were killed and each kidney, liver, intestine and heart were harvested from mice of each group at zero time, end of first two weeks and end of second two weeks were weighed and rapidly kept in formalin solution 10% and room temperature for histopathology examination. Body weights of mice of subjected groups were accurately weighted at zero time, end of each week along the experimental period.

Analysis & Investigation all the following estimations were carried out in the Central Laboratory for Food and Feed except histopathological studies which were carried out in Animal Health Research Institute. These estimations were taken place at the 3- interval periods "zero time, the end of the first interval and at the end of the

experimental period", except diets analysis which were conducted only after complete homogenization of their ingredients.

1) Estimation of minerals [calcium, iron, zinc and copper] according to AOAC, (2002) in both 5- prepared diets and mice hair by inductive coupled plasma ICP "optima 2000" as well as, in mice blood serum by zeman spectrometer "4100".

2) Diets analysis for determination of moisture content, crude protein, fiber and fat according to AOAC, (2000), ash obtained by AOAC, (1995) and phytic acid according to Wheeler and Ferrel, (1971).

3) Blood functions analysis for determination of alkaline phosphatase level according to Rick, (1990), estimation of urea by Fawcett and scott, (1960) and creatinine by Schirmeister, (1964). In addition to assess of HDL- C "High Density Lipoprotein Cholesterol level" by Friedewald *et al.* (1972) and LDL- C "Low Density Lipoprotein Cholesterol level" according to Levy, (1981).

4) Histopathological examination for the collected organs "liver, kidneys, intestine and heart" through the 3- interval periods according to the Bancroft *et al.* (1996).

RESULTS AND DISCUSSION

1- Assessment of 5- different diets:

Table 2. Estimation of some ingredients of Diets (g/100g diet):

Groups \ Ingredients	Fat	Carbohydrate (Starch & Sucrose)	Fiber	Protein	Ash	Moist.
A	4.89	ND	4.86	18.02	2.27	5.28
B	4.69	ND	5.07	17.70	2.03	4.51
C	4.66	ND	4.56	18.10	2.36	4.60
D	5.05	ND	5.51	18.90	2.48	5.19
E	4.97	ND	5.49	18.40	2.59	5.27

Table 3. Diets Analysis for Minerals and Phytic acid (mg/1000g diet):

Ingredients / Groups	MINERALS				Phytic acid
	Calcium	Iron	Zinc	Copper	
A	4595	41.06	09.60	5.79	Zero
B	4526	32.67	31.30	4.57	Zero
C	4858	40.65	60.00	4.23	Zero
D	4903	44.34	58.00	5.04	1497.0
E	4899	46.79	58.40	4.60	1505.4

Table 2 shows the analysis of the some ingredients of previously prepared 5-tested diets after complete homogenization of their ingredients according to the basal composition diets of table 1. Table 3 illustrates that, in spite of no zinc was added to the diet of mice fed zinc deficiency of group (A), the analysis reported that, this diet was contained 9.6 mg Zn / kg diet and this amount of zinc was obtained from the mixture of minerals and vitamins mixtures Stang *et al.* (2000). In addition, the diet of group (B) is the zinc controlled diet [31.3 mg Zn /kg diet as $ZnSO_4.7H_2O$] and the diet of group (C) is rich in zinc [60mg Zn /kg diet as $ZnSO_4.7H_2O$]. On the other hand, diets of groups (D) and (E) included phytic acid in concentration of 1497.0 and 1505.4 mg /kg diet respectively, as well as, diet (D) supplemented with zinc as $ZnSO_4.7H_2O$ in concentration of 58 mg/kg diet, while diet (E) was contained zinc in form of $ZnNa_2EDTA$ with concentration "58.4 mg / kg diet" as shown in table 3.

2- **Effects of zinc absorption on general appearance, body weight and diet consumption:**

Table 4. Estimation of the weight of food consumption and the Body weight of Mice:-

GROUPS / ITEMS	A	B	C	D	E
1- Food Consumption. (gm /day).					
1.1- first 2- weeks.	27.24 ± 2.00	28.36 ± 2.80	28.76 ± 3.50	29.60 ± 2.94	30.44 ± 3.63
1.2- second 2- weeks.	12.06 ± 1.06	25.60 ± 2.00	31.55 ± 2.14	18.86 ± 1.45	34.36 ± 3.80
2- Body Weight (gm).					
2.1- at zero time.	31.25 ± 0.55	31.25 ± 0.55	31.25 ± 0.55	31.25 ± 0.55	31.25 ± 0.55
2.2- after 2- weeks.	32.45 ± 0.25	32.37 ± 0.57	33.02 ± 0.92	26.42 ± 0.42	33.28 ± 1.28
2.3- after 4- weeks.	23.40 ± 0.40	33.87 ± 1.57	36.03 ± 1.03	22.45 ± 0.55	38.72 ± 1.12

The group of male mice fed zinc-deficient diet group (A) did not lose weight during the first 2- weeks, from the third week onward, a gradual loss of body weight occurred while mice of the group (D) [due to presence of dietary fiber of phytic acid in their diet], the loss of body weight occurred by the end of the second week as shown in table (4). Mice of groups (A) and (D) consumed lesser than the control due to loss of appetite, this is apparent in group (A) than group (D). By the end of the 4th- week, the mice body weights of groups (A) and (D) were lowered than that control mice group (B) and than that in zero time in spite of the *adlibitum* fed $ZnSO_4.7H_2O$ supplemented diet in group (D). These results are in agreement with those of Pang *et al.* (1992), ElzbietaI *et al.* (2001) and Daniel *et al.* (2003). The diets consumed by mice of group (C) and group (E) were very higher than that the control during the 4-weeks of the experimental period. This was accompanied by an increase in their weights which were highly increased than that of the control during the same period and of zero time. The consumption of diet and the body weight of mice reached to their maximum values in mice of group (E) fed *adlibitum* $ZnNa_2EDTA$ supplemented diet in the presence of dietary fiber of phytic acid as shown in table (4). Nearly 25% of the mice of zinc-deficiency groups (A) as well as (D) had diarrhea and were less active, lost hair, showed acrodermatitis and typical skin lesions on the tail and paws in agreement with the work of Pang *et al.* (1992), NRC, (1995) and ElzbietaI *et al.* (2001). No such lesions occurred in the other mice groups.

3- **The effect of interactions between zinc absorption and absorption of other minerals " calcium, iron and copper " in serum of blood of Swiss Webster out bred male mice in presence of dietary fiber and phytic acid:**

Table 5. Blood Serum Analysis for Minerals:

GROUPS / MINERALS	A	B	C	D	E
1- Calcium (mg/l) 1.1- at zero time. 1.2- after 2- weeks. 1.3- after 4- weeks.	104.00 ± 15.83 114.17 ± 10.17 122.00 ± 12.00	104.00 ± 09.00 111.96 ± 11.16 122.00 ± 12.00	104.00 ± 09.00 113.50 ± 06.50 123.50 ± 05.50	104.00 ± 09.00 106.33 ± 11.33 071.17 ± 07.17	104.00 ± 09.00 108.50 ± 12.50 074.17 ± 12.17
2- Iron (ugm /l) 2.1- at zero time. 2.2- after 2- weeks. 2.3- after 4- weeks.	39.50 ± 06.50 41.58 ± 06.08 47.97 ± 05.83	39.50 ± 06.50 42.51 ± 03.91 46.92 ± 06.68	39.50 ± 06.50 40.83± 03.83 48.50 ± 04.50	39.50 ± 06.50 39.83 ± 07.83 11.83 ± 08.83	39.50 ± 06.50 38.68 ± 06.92 19.00 ± 07.00
3- Zinc(ugm /l) 3.1- at zero time. 3.2- after 2- weeks. 3.3- after 4- weeks.	03.98 ± 00.38 05.34 ± 02.34 00.49 ± 00.49	03.98 ± 01.72 10.25 ± 01.45 16.23 ± 02.23	03.98 ± 01.72 17.70 ± 01.70 20.40 ± 03.48	03.98 ± 01.72 06.25 ± 00.35 02.55 ± 01.55	03.98 ± 01.72 20.50 ± 02.30 26.70 ± 01.72
4- Copper(ugm /l) 4.1- at zero time. 4.2- after 2- weeks. 4.3- after 4- weeks.	01.17 ± 00.25 02.57 ± 00.48 04.82 ± 00.30	01.17 ± 00.25 02.21 ± 00.23 03.06 ± 00.46	01.17 ± 00.25 00.94 ± 00.34 Nil	01.17 ± 00.25 00.89 ± 00.17 Nil	01.17 ± 00.25 04.15 ± 00.35 07.63 ± 00.53

As shown in table 5, calcium and iron levels in blood serum of mice were not affected by zinc deficient diet and zinc supplemented diets as in groups (A) and (C) with no concluded variation from their levels in blood of controlled mice group (B)

along the 4- weeks of the experimental period. On other hand, calcium and iron levels in blood of mice were affected by the presence of phytic acid as shown in supplemented mice groups (D) and (E). These results are agreement with that of Bolonnerdal, (2000) and supported by Lena *et al.* (2005), where the absorption of calcium and iron was highly and obvouisly reduced only by the end of the 4th - week of the experimental period [due to their depletion by the action of phytic acid of wheat bran content] as compared with the control group and zero time. The calcium content of the diet may, however, positively affect zinc absorption from phytate-containing meals via neutralizing the negative charges of phytic acid, it has been postulated that the formula [Ca] x ([phytate]/ [Zn]) ratio can be used as a predictor of zinc bio availability Fordyce *et al.* (1987) and Bolonnerdal, (2000), but presence the casein in the diet increases the inhibition of zinc absorption and decreases its utilization as concluded with Bolonnerdal, (2000).

Zinc level in mice blood serum of group (A) fed zinc deficient diet was slightly increased at the end of the 2- weeks of the experimental period than zero time, but lower than the control group along the same period, and was completely depleted by the end of the 4th – week. On the other hand, zinc level showed an increase in blood of mice groups (C) and (E) fed $ZnSO_4.7H_2O$ and $ZnNa_2EDTA$ supplemented diets respectively than level of zero time and control group and along the experimental period, especially in group (E) where zinc absorption record the maximum score in this group, in spite of the contained casein and phytic acid of their diet. This is attributed to the presence of EDTA that helps up take of zinc from the phytate- zinc –complex and form stronger complexes due to high binding constant between zinc and amino acids resulted from partially and completely digestion of casein. These results are in agreement with those obtained by Davidsson *et al.* (1994), Bolonnerdal, (2000) and Manjula *et al.* (2004). as well, mice fed casein based diet with phytic acid, Na_2EDTA was shown to improve zinc absorption, but had no effect in the absence of this inhibitory ligand Bolonnerdal, (2000). Contrary to about, mice group (D) fed $ZnSO_4.7H_2O$ supplemented diet due to the presence of dietary fiber of phytic acid, their blood serum zinc was decreased than control group along the 4- weeks period of the experimental.

Copper level in mice blood serum is reversibly proportional to zinc in blood due to the aggressive competition between zinc and copper absorption on the same cites of small intestine lumen Fosmire, (1990) and Jorge, (2003), these results were obviously

shown in mice blood levels of groups (A), (B) and (C) as shown in table (4). Even, the stability of the EDTA complexes whether zinc or copper was at the same gastric optimal P^H ($P^H = 4$) West and Sykes, (1960) increased the size of the competition between them. Also, the copper serum level was decreased by the end of the second 2- weeks of the experimental period in group (D) fed diet containing phytic acid. However, there was highly increase in copper serum level of mice of group (E) fed $ZnNa_2EDTA$ supplemented diet containing phytic acid than other groups and along the 4- weeks, with the increase of serum zinc level on using bran as source of fiber fortified with NaFeEDTA to improve the absorption of iron, these results are in agreement with those of Bolonnerdal, (2000).

The documented explanations for the undoubted effects of phytic acid on the absorption of the last nutrient metals were summarized in that, phytic acid molecule has a high phosphorous content "28.2 %" and chelating potential to form a wide variety of insoluble salts with di- and trivalent cations at neutral P^H. One mole of phytic acid can bind an average of 3-6 moles of Ca to form insoluble phytates at the P^H of the small intestine, formation of insoluble phytate makes both Ca and P unavailable. Zn, Cu and Fe can also be complexes, but Zn and Cu have the strongest binding affinity, this binding potentially renders these minerals unavailable for intestinal absorption Vohra *et al.* (1965).

4- Study the effect of interaction between zinc absorption and the absorption of other minerals "calcium, iron and copper" on hair mineral analysis of Swiss Webster out bred male mice in presence of dietary fiber (phytic acid):

Table 6 illustrates the hair content of minerals "Ca, Fe, Zn and Cu". Calcium in mice hair was not significantly affected by any zinc level in mice blood serum of groups [A, B and C], but affected by the phytic acid in diets of groups (D) and (E) through the experimental period compared with zero time in spite of the no change in Ca serum level after two weeks table 4.

Iron of mice hair, was not affected neither by feeding on zinc deficient nor by zinc supplemented diet along the 4- weeks of the experimental period as in hair of mice groups (A) and (C) respectively. But in presence of dietary fiber of phytic acid in diet as in groups (D) and (E), hair content of iron was significantly decreased along the 4- weeks than zero time and control as observed in table (6) in spite of no change in serum iron levels of these groups after the second week as shown in table (5).

Zinc of mice hair, was significantly increased along the 4- weeks of experimental period than zero time in all groups except groups (A) and (D) characterized by low bioavailability of zinc due to low zinc intake and presence of dietary fiber of phytic acid respectively, table 6.

Table 6. Hair Analysis of Minerals:

GROUPS / MINERALS	A	B	C	D	E
1- Calcium. (ppm)					
1.1- at zero time.	15.70 ± 1.20	15.70 ± 1.20	15.70 ± 1.20	15.70 ± 1.20	15.70 ± 1.20
1.2- after 2- weeks.	18.68 ± 1.82	18.91 ± 0.86	18.55 ± 1.45	12.84 ± 0.56	11.10 ± 1.01
1.3- after 4- weeks.	23.40 ± 0.90	23.70 ± 1.10	25.90 ± 1.67	09.68 ± 1.00	09.36 ± 0.66
2- Iron. (ppb)					
2.1- at zero time.	25.88 ± 2.90	25.88 ± 2.90	25.88 ± 2.90	25.88 ± 2.90	25.88 ± 2.90
2.2- after 2- weeks.	27.60 ± 1.15	26.60 ± 1.78	27.80 ± 0.55	17.10 ± 0.74	19.00 ± 0.05
2.3- after 4- weeks.	30.91 ± 2.58	29.41 ± 1.20	30.18 ± 1.35	09.80 ± 1.00	11.15 ± 0.74
3- Zinc. (ppb)					
3.1- at zero time.	37.29 ± 4.46	37.29 ± 4.46	37.29 ± 4.46	37.29 ± 4.46	37.29 ± 4.46
3.2- after 2- weeks.	26.83 ± 1.80	40.14 ± 3.02	49.50 ± 2.90	26.14 ± 0.86	52.46 ± 2.14
3.3- after 4- weeks.	10.55 ± 0.90	46.07 ± 1.06	55.76 ± 3.98	19.32 ± 0.70	68.53 ± 1.50
4- Copper. (ppb)					
4.1- at zero time.	07.23 ± 0.92	07.23 ± 0.92	07.23 ± 0.92	07.23 ± 0.92	07.23 ± 0.92
4.2- after 2- weeks.	09.89 ± 0.08	08.74 ± 0.08	06.65 ± 0.76	03.40 ± 0.04	10.41 ± 0.85
4.3- after 4- weeks.	12.80 ± 0.02	10.40 ± 0.12	02.86 ± 0.90	00.80 ± 0.37	12.90 ± 0.78

From tables 5 and 6 copper absorption in mice blood serum was a good indicator for prediction of mice hair content of copper where groups of high serum copper (A) and (E) have high content of copper in their hair and vice versa group (C). These reflect the effect of the competition between zinc and copper absorptions into blood Fosmire, (1990) on the copper content of hair.

5- Weights, specific blood functions and the histopathological studies of Swiss Webster out bred male mice organs:

Tables 7 and 8 show the variation in weights of mice organs of the tested groups from zero time and along the 4- weeks of experimental period and their corresponding some functions:

5-1- liver: liver weights in zinc deficiency mice of both group (A) due to low zinc intake and group (D) due to presence of dietary fiber of phytic acid were reduced along the 4- weeks of experimental period as compared with the control group (B), while the mice liver weights of zinc supplemented groups (C) of $ZnSO_4.7H_2O$ and (E) of $ZnNa_2EDTA$ complex were increased than that of zero time and little rise than the control along the experimental period, but the mice liver weights of zinc supplemented

groups (D) of $ZnSO_4.7H_2O$ and in presence of phytic acid in their diets were lower than that in both mice of group (A) and controls, these results are in agreement with ElzbietaI *et al.* (2001). On the other hand, the alkaline phosphatase level which is one of the liver indicators of functions and the strongest indicator for zinc level in the blood was increased by increase in zinc absorption into mice blood as shown in each of the tables (5) and (8), these results are in agreement with Nicola *et al.* (2004). Group (D) was the lowest of the experimental groups in alkaline phosphatase level than control, on the other side, alkaline phosphatase levels in mice blood of groups (B), (C) and (E) were gradually increased especially in group (E) in accordance to zinc absorption.

The aggressive competition between the absorption of zinc and copper reflected its results on the cholesterol levels in the blood serum of mice, where the increase of copper absorption lead to increase the HDL- C "good cholesterol" and lowered the LDL-C "bad cholesterol" [Groups A and E]. The reverse were obtained in mice of groups (C) and (D) where the increase of zinc absorption inhibited the absorption of copper in group (C), while the presence of bran as dietary fiber containing phytic acid in group (D) decreased the bio availability and the utilization of copper which resulted in reduction of the copper level in mice blood, as shown in table (5) and decreased the HDL- C and increased the corresponding LDL- C as shown in table (8), all these results are in agreement with Reiser *et al.* (1987), Sandstead ,(1995) and Jorge, (2003)

Groups (A) and (D) showed severely congested blood vessels with per vascular aggregation of inflammatory cells of mice liver in zinc deficiency, also Parenchyma focal aggregations were observed by the end of the 2nd- week, as well as sub capsular hemorrhages were detected and the hepatocytes exhibited cloudy swelling as shown in fig (2). By the end of the 4th- week, the majority of liver cells completely lost their cytoplasmic structure as shown in fig 3. No definite lesions were showen in liver of other groups, these results are in agreement with ElzbietaI *et al.* (2001) and Dae *et al.* (2003).

5-2- Small intestine: In mice of zinc deficient groups (A) and (D) only, by the end of the 4th- week of experimental period, the intestine weight of mice group (A) were significantly decreased, while this decrease was observed also after 2- weeks in mice of group (D). There was increase in intestines weight of mice group (E). Intestine weights of supplemented mice group (C) did not vary from control along the Exp. Period and these results are in agreement with ElzbietaI *et al.* (2001).

On the other hand, by the end of 2nd- week of exp. period, the blood vessels of lamina propria were congested and inflammatory cells were detected in small intestine of mice groups (A) and (D) as shown in fig (4). With time progression and by the end

of the 4th- week of the exp. period, the epithelial lining of small intestine of Zn-deficiency mice groups (A) and (D) suffered from autolysis, the muscular layers showed degenerative, changes, severely congestion and vacuolization as shown in fig (5), these results are in agreement with Marilyn and Kristine, (2000) and Alberto *et al.* (2003).

5-3- Kidneys: The study did not show any significant difference between weights of mice kidneys of all experimental groups than control group and along the 4- weeks of experimental period [table 7]. However, there were highly increase in the urea of mice blood of groups (C) and (E) and especially group (E) than control mice of group (B) and zinc deficiency mice group (A) along the 4- weeks of experimental period especially the last 2- weeks shown in table 8. In the same time, there was highly evolution of the creatinine in the serum blood of mice of the mice groups (C) and (E) than control mice group, while the creatinine of group (A) of zinc deficiency, decreased than control group as shown in table 8. The explanation of these results may be due to the high concentration of zinc levels 60mg and 58.4mg/kg diet of the diets of mice groups (C) and (E) respectively, as shown in table 3, which might affect the kidney functions.

5-4- Heart there were significant variations in heart weights for any mice groups along the 4- weeks of the exp. period as shown in table (7).

Most of the main of coronary blood vessels of mice of groups (C) and (D) were dilated and engorged with blood and the cardiac muscles existed myomalacia by the end of the 4th- week. These observed lesions may be due to the increase in the blood cholesterol of LDL- C " bad cholesterol " in mice blood as a result of inhibition of the absorption of copper because of the higher absorption of zinc into mice blood serum as in group (C) and the action of dietary fiber of phytic acid as in group (D), which lead in the end to lowering the utilization of copper and increasing the LDL- C " bad cholesterol ". On the other hand, the explanation of these results may be due to the high concentration of zinc levels 60mg /kg diet in the diet of mice group (C), as shown in table (3), which may stop competition between Zn- and Cu- absorption, but in case of mice group (D), the phytic acid content in their diet was very sufficient to inhibit the Cu- absorption and its utilization. These results are in agreement with Reiser *et al.* (1987), Fosmire, (1990) and Sandstead, (1995).

Table 7. Organs weight of the experimental mice:-

Weight of Organs (gm) \ GROUP	A	B	C	D	E
1- Liver.					
1.1- at zero time.	1.80 ± 0.10	1.80 ± 0.10	1.80 ± 0.10	1.80 ± 0.10	1.80 ± 0.10
1.2- after 2-weeks.	1.70 ± 0.23	2.00 ± 0.27	2.00 ± 0.18	1.72 ± 0.10	2.02 ± 0.12
1.3- after 4-weeks.	1.98 ± 0.13	2.18 ± 0.23	2.26 ± 0.17	1.84 ± 0.20	2.42 ± 0.10
2- Kidneys (Right /LEFT).					
2.1- at zero time.	0.23 ± 0.05	0.25 ± 0.05	0.25 ± 0.05	0.27 ± 0.07	0.25 ± 0.05
	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.05	0.28 ± 0.08	0.25 ± 0.05
2.2- after 2- weeks.	0.30 ± zero	0.30 ± zero	0.30 ± zero	0.30 ± zero	0.32 ± 0.08
	0.30 ± zero	0.30 ± zero	0.30 ± zero	0.30 ± zero	0.32 ± 0.07
2.3- after 4- weeks.	0.28 ± zero	0.30 ± zero	0.32 ± zero	0.30 ± zero	0.35 ± zero
	0.28 ± 0.07	0.30 ± 0.10	0.32 ± zero	0.30 ± zero	0.35 ± 0.08
3- Small Intestine.					
3.1- at zero time.	1.75 ± 0.45	1.75 ± 0.45	1.75 ± 0.45	1.75 ± 0.45	1.75 ± 0.45
3.2- after 2- weeks.	1.77 ± 0.47	1.93 ± 0.43	2.00 ± 0.48	1.58 ± 0.38	2.17 ± 0.37
3.3- after 4- weeks.	1.58 ± 0.55	2.03 ± 0.43	2.27 ± 0.47	1.52 ± 0.32	2.22 ± 0.32
4- Heart.					
4.1- at zero time.	0.19 ± 0.04	0.19 ± 0.04	0.19 ± 0.04	0.19 ± 0.04	0.19 ± 0.04
4.2- after 2- weeks.	0.20 ± zero	0.19 ± zero	0.20 ± zero	0.20 ± zero	0.20± zero
4.3- after 4- weeks.	0.20 ± zero	0.20 ± zero	0.19 ± zero	0.20 ± zero	0.20 ± zero

Table 8. Estimations of specific organs functions in mice serum:

Mice Groups / Functions	A	B	C	D	E
Alkaline Phosphatase Levels (unit/I):-					
At Zero Time	087 ± 21	087 ± 21	087 ± 21	087 ± 21	087 ± 21
After 2- Weeks	041 ± 27	095 ± 13	123 ± 16	039 ± 02	117 ± 13
After 4- Weeks	025 ± 06	113 ± 06	148 ± 08	022 ± 07	164 ± 17
Cholesterol Levels:- (mg/dl) 1- Total Cholesterol.					
At Zero Time	151 ± 07	151 ± 07	151 ± 07	151 ± 07	151 ± 07
After 2- Weeks	147 ± 03	149 ± 04	154 ± 05	157 ± 02	145 ± 11
After 4- Weeks	143 ± 04	148 ± 03	156 ± 06	160 ± 04	140 ± 09
2- HDL- Cholesterol.					
At Zero Time	078 ± 04	078 ± 04	078 ± 04	078 ± 04	078 ± 04
After 2- Weeks	082 ± 04	080 ± 05	074 ± 06	071 ± 06	082 ± 08
After 4- Weeks	085 ± 03	083 ± 02	071 ± 04	067 ± 03	088 ± 03
3- Triglycerides.					
At Zero Time	110 ± 05	110 ± 05	110 ± 05	110 ± 05	110 ± 05
After 2- Weeks	085 ± 05	095 ± 30	135 ± 25	160 ± 40	085 ± 00
After 4- Weeks	065 ± 10	080 ± 25	140 ± 00	150 ± 25	055 ± 15
4- LDL- Cholesterol.					
At Zero Time	051 ± 04	051 ± 04	051 ± 04	051 ± 04	051 ± 04
After 2- Weeks	048 ± 03	050 ± 05	053 ± 04	054 ± 04	046 ± 03
After 4- Weeks	045 ± 03	049 ± 06	057 ± 02	063 ± 06	041 ± 02
Kidney Functions:- (mg/dl) 1- Creatinine.					
At Zero Time	0.18 ± .01	0.18 ± .01	0.18 ± .01	0.18 ± .01	0.18 ± .01
After 2- Weeks	0.18 ± .01	0.19 ± .03	0.90 ± .08	0.30 ± .04	0.95 ± .19
After 4- Weeks	0.19 ± .01	0.21 ± .01	1.80 ± .11	0.49 ± .07	2.05 ± .22
2- Urea.					
At Zero Time	4.50 ± .03	4.50 ± .03	4.50 ± .03	4.50 ± .03	4.50 ± .03
After 2- Weeks	4.70 ± .03	4.70 ± .04	7.20 ± .18	5.10 ± .04	7.90 ± .08
After 4- Weeks	4.90 ± .08	4.90 ± .03	9.50 ± .14	6.20 ± .11	10.5 ± .14

CONCLUSION

Calcium and iron were not affected by neither zinc deficiency nor zinc supplementation in serum blood of Swiss Webster out bred male mice. Due to the aggressive competition between zinc and copper absorption on the same cites of small intestine lumen, copper absorption was positively affected by zinc deficient diet and recorded significant increasing in HDL- C "good cholesterol" and was negatively affected by zinc supplementation [as hydrated zinc sulfate "ZnSO$_4$ .7H$_2$O"] and recorded highly value of LDL- C "bad cholesterol". While, when mice were fed *adlibitum* chelated zinc supplemented diets as zinc di sodium ethylene di amine tetra acetate "ZnNa$_2$EDTA", it partially dissociated this chelated complex in the gut and allowed zinc to be absorbed, also it re- chelated with copper, protected it from action of phytic acid complexation allowed the copper to be completely absorbed.

Phytate is known to form complex with nutritional metals" Ca, Fe, Zn and Cu". Thus, the unavailable phytate metallic nutrients complex can not be utilized and were excreted. Zinc may be the trace element whose bio availability is most influenced by phytate, thus, the chelation of zinc by EDTA raised the utilization and bio-availability of zinc in the presence of bran as dietary fiber containing phytic acid. Increasing the calcium concentration in the diets could have an effect on zinc absorption by neutralization the negatively charges of the phytate that inhibit zinc uptake.

Hair provides a unique biopsy type of reading of metabolic activity. Minerals are shifted from the tissues to maintain blood levels, this means that deficiencies or excesses often show up earlier in the hair than the blood of Swiss Webster out bred male mice. Hair analysis for minerals, gives a good indication of actual mineral levels while blood levels may still be in the normal range, thus, hair analysis gives clues concerning relative deficiencies and can be effectively used for biological monitoring of the highest priority nutritional metals. On the other hand, supplementation with $ZnNa_2EDTA$ can improve the zinc and copper status of the subjects and increases their utilization from the supplemented diet especially in the presence of anti- nutritional factors as dietary fiber of phytic acid without any effects on the bio availability or absorption of calcium and iron. Finally, the alkaline phosphatase level is one of the liver functions and the strongest closed indicator for zinc level in the blood serum. By the end of the 4th- week, also, from the histopathlogical studies, the majority of liver cells completely lost their cytoplasmic structure in mice of zinc deficiency due to low intake of zinc or presence of dietary fiber of phytate. Therefore we recommended by fortifying the floor of the balady bread with zinc in EDTA form as strategy for the governorates which their villages suffered from signs and symptoms of zinc deficiency.
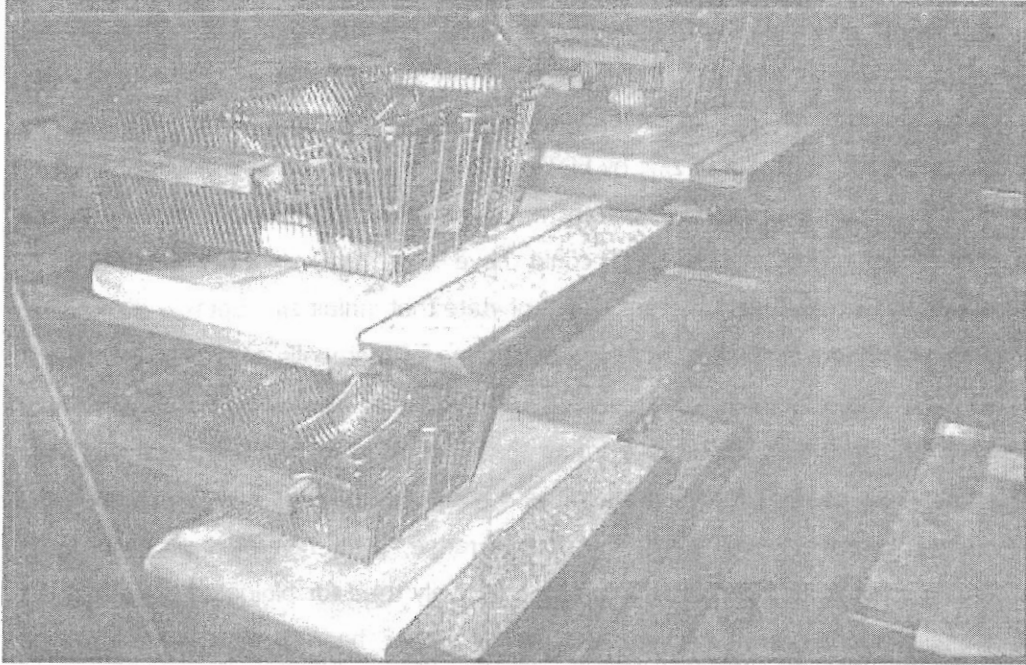
ACKNOWLEDGMENTS

Fig 1. Mice in these experiments were housed individually in stainless steel cages were previously randomly distributed on 5- subjected experimental groups.
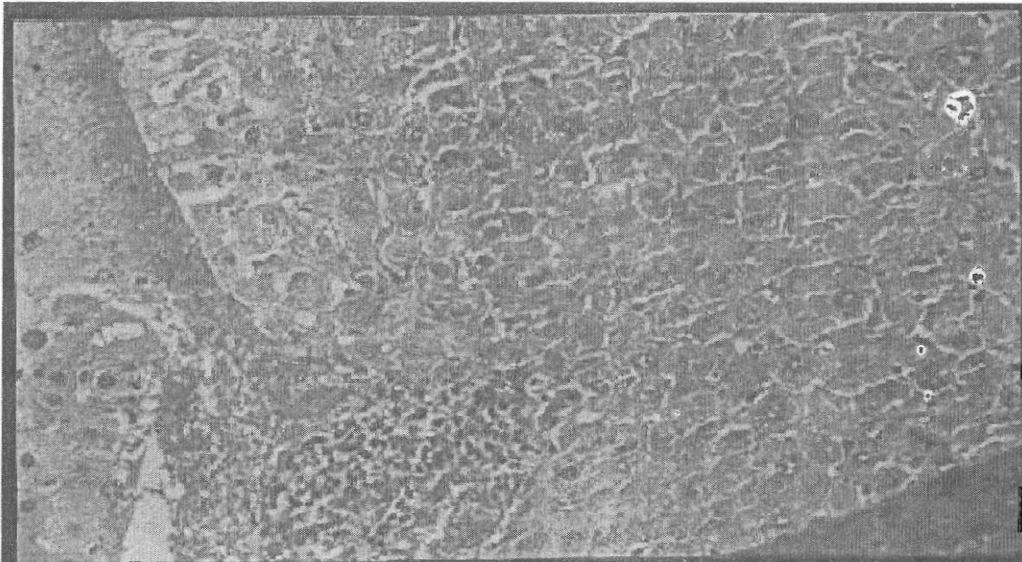

Fig 2. liver of Swiss Webster out bred male mice showing perivascular aggregation of inflammatory cells in zinc deficiency mice groups (A) and (D) by the end of the 2nd-week.
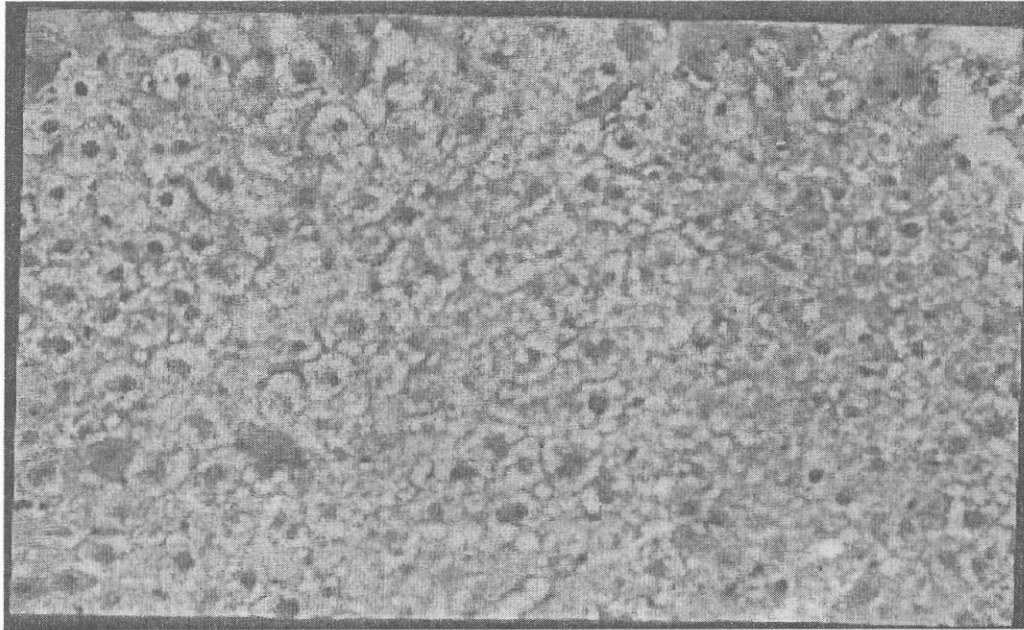
H & E   × 250

Fig 3. liver of Swiss Webster out bred male mice showing hepatocytes with cytoplasmic structure loss in Zn- deficient   mice groups (A) and (D) by the end of the 4th- week.
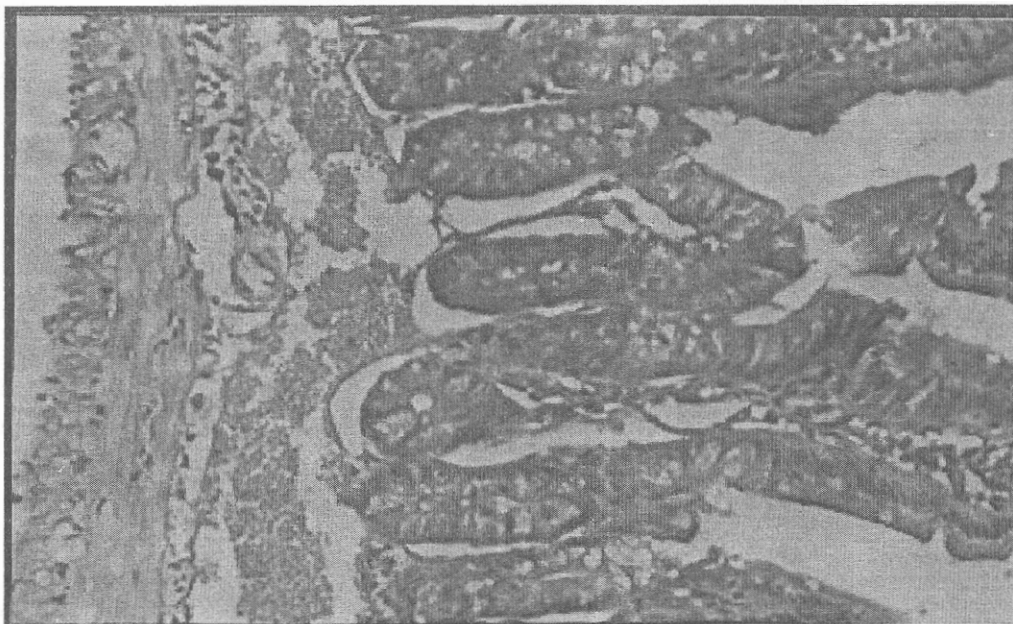
H & E × 250

Fig (4) small intestine of Swiss Webster out bred male mice of zinc deficiency of groups (A) and (D) showing congested blood vessels inflammatory cells by the end of 2nd- week of exp. Period.

H & E   × 250

Fig 5. small intestine of Swiss Webster out bred male mice of zinc deficiency groups (A) and (D) showing muscular layer vacuolization by the end of 4th- week .
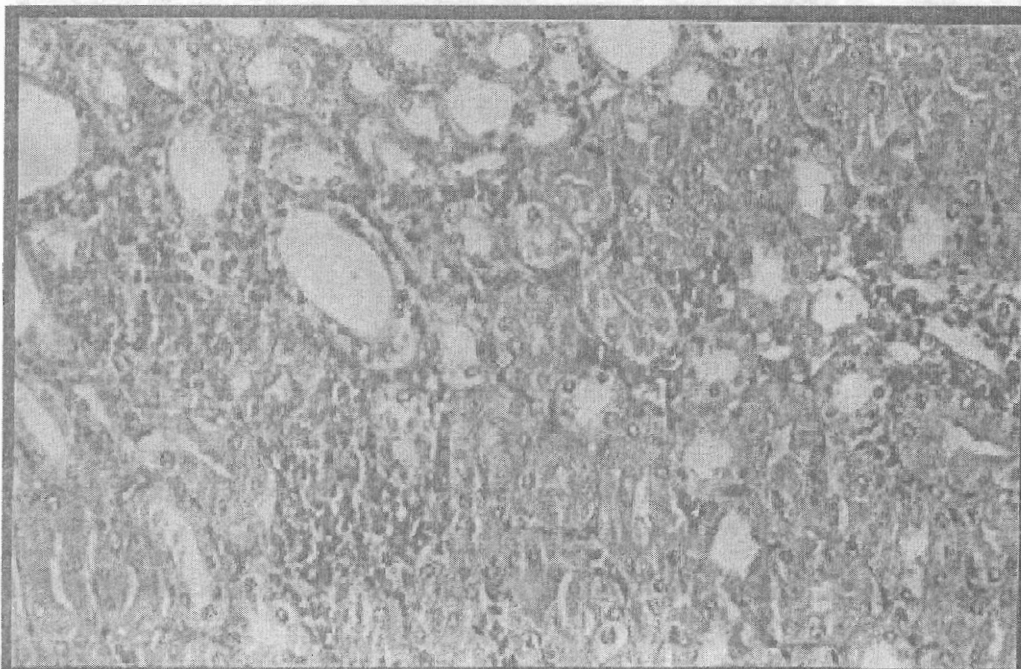H & E   × 400

Fig 6. kidney of mice of groups(C) and (E) showing inter tubular inflammatory cells infiltration by the end of the 4th- week of the exp. period.
H & E   × 250

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دراسة بيئية لبعض العوامل المؤثرة على الأتاحة الحيوية للزنك

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تم إجراء الدراسة على فئران من نوع ال Swiss Webster Mice عمر خمسة أسابيع منتقاه وتم وضعها منفردة فى أقفاص من الحديد المقاوم للصدأ(Stainless Steel) فى معمل البيولوجى التى تتراوح درجة حرارته بين ٢٢ إلى ٢٤ درجة مئوية وفى رطوبة نسبية مداها ٤٥– ٥٥ %.

تم عمل التحليلات لخمس أنواع من الوجبات المقدمة للفئران (بعد تجنيس تلك الوجبات) وقد كانت تلك الوجبات مختلفة فقط فى تركيز ونوع مصدر الزنك وكذلك مصدر الألياف مع ثبات بقية المغذيات الأخرى دون أى تغير لا فى التركيز أو المصدر كما يلى:-

١ – مجموعة الفئران (أ) مغذاه على وجبة بها نقص فى المحتوى الزنكى وتحتوى على ٩،٦ ملليجرام زنك / كيلو جرام وجبة ومصدر الزنك بها هو كبريتات الزنك ومصدر الألياف هو السيليلوز وتركيز حامض الفيتيك فى هذه الوجبة = صفر.

٢ – مجموعة الفئران (ب) مغذاه على وجبة محتواها الزنكى حسب الإحتياجات العمرية فقط للفئران وتحتوى على ٣١،٣ملليجرام زنك / كيلو جرام وجبة ومصدر الزنك بها هو كبريتات الزنك المائية ومصدر الألياف هو السيليلوز وتركيز حامض الفيتيك فى هذه الوجبة = صفر وهذه المجموعة من الفئران هى مجموعة الكونترول (Controls).

٣ – مجموعة الفئران (ج) مغذاه على وجبة محتواها الزنكى أكثر من الإحتياجات العمرية للفئران وتحتوى على ٦٠،٠ملليجرام زنك / كيلو جرام وجبة ومصدر الزنك بها هو كبريتات الزنك المائية ومصدر الألياف هو السيليلوز وتركيز حامض الفيتيك فى هذه الوجبة = صفر.

٤ – مجموعة الفئران (د) مغذاه على وجبة محتواها الزنكى أكثر من الإحتياجات العمرية للفئران وتحتوى على ٥٨،٠ملليجرام زنك / كيلو جرام وجبة ومصدر الزنك بها هو كبريتات الزنك المائية ومصدر الألياف هو ردة القمح (Wheat bran) المستخدمة فى خبيز العيش البلدى

ومخبوزات أخرى وتركيز حامض الفيتيك فى هذه الوجبة = ١٤٩٧٫٠ ملليجرام / كيلو جـرام وجبة.

٥- مجموعة الفئران (ه) مغذاه على وجبة محتواها الزنكى أكثـر مـن الإحتياجـات العمريــة للفئران وتحتوى على ٥٨٫٤ ملليجرام زنك / كيلو جرام وجبة ومصدر الزنك بها هو زنك ثنائى الصوديوم ثنائى الأمين رباعى الأسيتات (ZnNa₂EDTA) ومصدر الألیــاف هـو ردة القمـح (Wheat bran) المسنخدمة فى خبيز العيش البلدى ومخبوزات أخرى وتركيز حامض الفيتيـك فى هذه الوجبة = ١٥٠٥٫٤ ملليجرام / كيلو جرام وجبة.

وقد تم تصميم التجربة لإختبار التفاعلات التداخلية بين عنصر الزنك والعناصـر المعدنيـة المغذية الأخرى مثل الكالسيوم والحديد والنحاس، وكذلك لدراسة تأثير حامض الفيتيك على إمتصاص الزنك ، كما تم أيضا دراسة تعزيز إمتصاص الزنك فى وجود حامض الفيتيـك بإسـتخدام مصـدر مخلبى له مثل زنك ثنائى الصوديوم ثنائى الأمـين ربـاعى الأسـيتات (ZnNa₂EDTA) وإضـافته للوجبات.

وكانت مدة التجربة ٤- أسابيع.

وأكدت نتائج الدراسة أن مستوى كل من الكالسيوم والحديد فى سيرم دم الفئران لم يتأثـر سواء بنقص أو بزيادة تركيز الزنك فى الوجبات المعطاة لذكور الفئران المختارة.

أظهرت النتائج أيضا أنه نتيجة للمنافسة الشديدة بين إمتصاص كل من الزنك والنحاس على نفس أماكن الأمتصاص فى الأمعاء الدقيقة، فإن مستوى النحاس فى سيرم دم الفئران يتأثـر إيجابيـا بشـدة بنقص الزنك فى الوجبات المقدمة للفئران وكذلك يكون مصاحبا له زيادة ملحوظة فى الكوليسـترول النافع للصحة(HDL- C) ويتأثر سلبيا بشدة من تناول الوجبات الغنية بالزنك والتى مصدر الزنك بهـا كان كبريتــات الزنك المائبة والذى يكون مصحـوبا بزيادة نسبة الكوليسـترول الضـار بالصحـة (LDL- C).

أما تناول الفئران للوجبات المدعمة بالزنك فى صورته المخلبية زنك ثنائى الصوديوم ثنـائى الأمين رباعى الأسيتات (ZnNa₂EDTA) فقد سجل زيادة ملحوظة فى مستويات الإمتصاص لكل من الزنك والنحاس فى سيرم دم فئران المايس.

ومن المعروف إن حامض الفيتيك الموجود بردة القمح المستخدمة فى خبيز العيش البلدى الـذى يتناوله القاعدة العريضة من الناس والتى تستخدم أيضا بكميات كبيرة فى صناعة عيش السن الـذى يتناوله عدد كبير من الناس من الراغبين فى إنقاص وزنهم أو من مرضى السكر إلا إن هذا الحامض يكون أملاح صلبة معقدة مع العناصر المعدنية المغذية (الكالسيوم والحديد والزنك والنحـاس) وهـذه الأملاح لا تذوب فى الأوساط البيولوجية حتى يسهل إمتصاص هذه العناصر وتخرج هذه الأمـلاح بواسطة البراز وبالتالى لايمكن الأستفادة من هذه العناصر.

إن تحليل الشعر لمحتواه المعدنى يعطى مؤشر واضح وجيد لمستويات المعادن الموجودة بالجسم، بينما لو أخذنا تحليل الدم من حيث مستوى تلك المعادن فى الجسم فأن النتائج كانت غير متفقة مع حقيقة هذه المستويات وذلك ربما بسبب إن أماكن الأيض تكون دائما الأنسجة (الشعر) وليس الدم وإن المعادن تنتقل من الأنسجة للدم محافظة على مستوى تلك المعادن بالدم، وربما بسبب ذلك فإن نقص أو زيادة تلك المعادن يظهر مبكرا فى الشعر عن الدم وإن تحليل الشعر هو وسيلة فعالة لمعرفة المستويات الحقيقية للمعادن بالجسم.

ونوصى فى هذا البحث بتدعيم الدقيق المستخدم فى صناعة الخبز البلدى بالزنك المخلبى " زنك ثنائى الصوديوم ثنائى الأمين رباعى الأسيتات (ZnNa$_2$EDTA)" وذلك فى مخابز المحافظات التى يعانى سكان قراها من علامات وأعراض نقص الزنك.