BIOLOGICAL STUDIES ON FLAT BREAD FORTIFIED WITH DIFFERENT SOURCES OF ZINC

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ABSTRACT

Hypozincemic rats fed on flat bread supplemented with different sources of zinc, i.e. natural (20% soy flour) or chemical (ZnO or $ZnSO_4$ at 8 mg/100 g flour) compared with control (100% wheat flour 72%). Biological evaluation of the experimental rats such as: body weight gain, weight of organs, glucose level, serum alkaline phosphatase, T_3 and T_4, serum testosterone, zinc in serum and zinc in tibia were estimated. The data showed that feeding flat bread supplemented with different sources of zinc increased the body weight and weight of organs. Chemical sources of zinc decreased glucose level compared with natural source. Zinc supplementation increased the level of T_3, T_4 and serum testosterone in rats. Zinc in serum and tibia were increased by zinc supplementation.

Keywords: wheat flour, soy flour, zinc, hypozincemic rats, glucose level, serum alkaline phosphatase, T_3, T_4, serum testosterone.

INTRODUCTION

Zinc is an essential trace element that is involved in more enzymatic reactions than any other minerals. Zinc is present in every cell and occurs in the second largest amounts in the body next to iron. The element is well known to protect the immune system and fight disease. It is crucial for the proper function of many hormones, including insulin, growth hormone, sex hormones, and hormones of the thymus gland (Murray, 1996). Zinc also plays a great role in male reproduction including sperm formation and motility, and hormone metabolism. Zinc levels are typically much lower in infertile men, and a zinc deficiency is associated with decrease of testosterone hormone levels and sperm counts. Zinc assists in wound healing because it is required for protein synthesis and cell growth. Zinc is also beneficial for internal wounds including ulcers. Zinc is used in the treatment of liver cirrhosis and alcoholism. Therefore, zinc supplementation should be used in the treatment of alcoholism. On the other hand, zinc is also indicated in diabetes because it has a regulatory effect on blood sugar levels (Murray, 1998).

Arreola *et al.* (1993) reported that oral zinc administration increased plasma levels of TSH, T_3, T_4 and zinc in uremic patients under peritoneal dialysis, who had been zinc-deficient. Zinc deficiency was first described in Egyptian and Iranian adolescents more than 30 years ago and continues to be a common health problem for children living in both developing and developed countries. Such deficiency is known to impair immune function and to retard growth in children. But its impact on brain function and mental performance has been demonstrated only recently. Excessive zinc consumption can impair immune function, and it is not recommended to take

dosages higher than 150 milligrams daily for more than 1 week (Murray, 1998). Zinc deficiency was shown to impair the metabolism of thyroid hormones. Kralik *et al.* (1996) reported that male Sprague-Dawley rats fed low-zinc diet for 40 days showed decreased serum concentrations of T3 and free T4 by approximately 30% compared with those fed adequate-zinc diet.

In many diets, cereals make a greater contribution than any oilier food groups to both energy and protein content. The Middle Eastern countries depend mainly on wheat. About two thirds of the available protein comes from wheat in the form of bread. In fact, soybean products are an important low-cost source of proteins, minerals, phosphorus and vitamins. Furthermore, soybean products play an important role in health (Messina, 1995). Zinc content in wheat flour (72%) is 0.3 – 0.42 mg/100g (Abd EL-Mottaleb, 2001) and in defatted soybean flour is 6.8 mg/100g (Bakry, 1997).

The aim of this study is to enrichment of flat bread with Zn either with natural or chemical sources. Its effect on some growth factors, enzymes and hormones of the experimental animals, was studied.

MATERIALS AND METHODS

Materials:

Wheat flour: American wheat flour (72% extraction rate) was obtained from South Cairo Mills Company, Fysal, Giza, Egypt.

Low fat soybean flour: was obtained from Soybean Pilot Plan, Food Technology Research Institute (FTRI), Agricultural Research Center, Giza, Egypt.

Biological and Baking ingredients: Salt, sugar and yeast were obtained from super market, Giza, Egypt.

Minerals and Vitamins: Zinc salts and the other minerals mixture used in the present study were obtained from Al- Nasr-Pharmaceutical Chemical Company, and vitamins mixture were obtained from Sigma Chemical Company.

Available drinking water: The water used to dink rats was redistilled and deionized by treatment redistilled water with mixed bed (anion and cation exchange resin).

Experimental animals: Forty weanling albino male rats (weighed 30 – 40g) were obtained from the experimental animal house, Crops Technology Department, FTRI, Giza, Egypt.

Methods:

Baking of flat Bread: Flat bread loaves were prepared using the method of Faridi and Rubenthaler (1984). The fortified bread was produced by substitution of 20% soy flour as natural sources of Zn, while ZnO or $ZnSO_4$ was added separately at the level of 8 mg/100g flour.

Biological evaluation:

Experimental design: The animals (40 weanling male albino rats) were housed in stainless steel cages at room temperature (25±2°C) and fed on

hypozincemic induced diet and redistilled deionized water for two weeks (adaptation period). After this period, animals (40 rats) were divided into three main groups and the third group divided into 6 subgroups as following scheme:

(Group I): Rats fed on the basal diet. (n= 5).

(Group II): Rats fed on the free zinc basal diet (hypozincemic induced diet) (n= 5).

(Group III): Rats were divided into 6 subgroups as follow: (n= 5).

- **(Subgroup 1):** Rats fed on the flat bread prepared from wheat flour only.
- **(Subgroup 2):** Rats fed on flat bread supplemented with 20% soy flour.
- **(Subgroup 3):** Rats fed on flat bread fortified with $ZnSO_4$ (80mg Zn/Kg diet).
- **(Subgroup 4):** Rats fed on flat bread fortified with ZnO (80mg Zn/Kg diet).
- **(Subgroup 5):** Rats fed on flat bread supplemented with 20% soy flour and fortified with $ZnSO_4$ (80mg Zn/Kg diet).
- **(Subgroup 6):** Rats fed on flat bread supplemented with 20% soy flour and fortified with ZnO (80mg Zn/Kg diet).

The basal diet, minerals and vitamins mixtures were prepared as recommended by A.O.A.C. (2000). During the experimental period, the rats were weighed and the blood sample was obtained from the orbital plexus of each rat. At the end of the experiment, the final weight of body and weight of organs were recorded. Blood samples were obtained and centrifuged to separate the serum. Then, the serum was frozen kept until analysis.

Biochemical analysis:

(1) Determination of serum glucose:
Blood glucose was determined according to the procedure of Trinder (1969).

(2) Determination of serum alkaline phosphatase activity:
The activity of alkaline phosphatase activity of serum was determined according to the method of Kind and King (1954).

(3) Determination of T_3 and T_4:
Serum 3, 5, ´3 triiodothyronine (T_3) and tetraiodothyronine (T_4) were determined according to the method of Wayne (1998).

(4) Determination of serum testosterone:
Serum testosterone of rats was determined according to the method of Chen *et al.* (1991).

(5) Determination of zinc in tibia and serum:
Zinc in tibia was determined according to the method outlined in A.O.A.C. (2000), and zinc of serum was determined according to the modified method of Makino and Takahara (1981).

Statistical analysis of the data:

Statistical analysis was carried out according to Fisher (1970). The statistical package for social science Anon (1999) program version 10 was used for all analysis.

RESULTS AND DISCUSSION

Effect of feeding flat bread supplemented with different sources of zinc on body weight gain, feed intake and feed efficiency:

Body weight gain, feed intake and feed efficiency of hypozincemic rats fed on basal diet, basal diet free of zinc (-Zn), flat bread made of wheat flour 72% or flat bread supplemented with soy flour and different sources of zinc are summarized in Table (1). From data, it is clear that different supplements of flat bread showed a significant increase in body weight gain when compared with hypozincemic diet and flat bread made of wheat flour only. Feed efficiency also followed the same trend (as a result of feed intake and body weight gain), where increased from 0.15 (basal diet (free Zn)) to 0.28 (flat bread supplemented with 20% soybean flour). The effect of different sources of zinc on body weight may due to the role of zinc on growth hormone (GH), T_3 and T_4 (Kaji *et al.*, 1998). Results are in accordance with those obtained by El-Hendy *et al.* (2001) who showed that zinc deficiency has negative effect on growth rate.

Effect of feeding flat bread supplemented with different sources of zinc on organs weight of rats:

The weight of organs and its ratio to body weight of experimental animals fed different formulas of flat bread containing Zn were given and recorded in Table (2). In hypozincemic rats, liver weight decreased but the ratio of liver weight / body weight increased compared with that fed on basal diet and flat bread. Feeding hypozincemic diet reduced testes weight, but in all flat bread groups, the weight of tests increased. It's clear that the general trend of liver weight was similar to brain, kidney, spleen and heart. On the other hand, the weight of tibia was increased with the increasing body weight.

The lowest value of tibia weight was in rats fed on basal diet (free Zn), but the highest weight of tibia was recorded by rats fed on flat bread supplemented with soy flour.

Effect of feeding flat bread supplemented with different zinc sources on glucose level and serum alkaline phosphatase activity in hypozincemic rats:

The effect of feeding flat bread supplemented with different sources of zinc on serum glucose level and serum alkaline phosphatase activity are reported in Table (3). From data, feeding rats on flat bread supplemented with chemical sources decreased serum glucose level compared with feeding on basal diet. This decrease may be due to the role of zinc in the assemble of insulin monomers to a dimeric form (Chausmer, 1998). The highest value of serum glucose level was detected at feeding on flat bread supplemented with 20% soy flour and 20% soy flour + $ZnSO_4$.

T1

t2

On the other hand, the data showed a highly significant decrease in serum alkaline phosphatase activity of rats fed flat bread supplemented with the different sources of Zn (except ZnSO4) compared with those fed on basal diet or basal diet free Zn. There was no significant difference between treatments containing soy flour or soy flour and $ZnSO_4$ compared with other treatments. These results are in agreement with the results obtained by Naber *et al.* (1996).

Table (3): Effect of feeding flat bread supplemented with different sources of zinc on glucose level and serum alkaline phosphatase activity of zinc deficiency rats

Diet	Supplements	Glucose		Alkaline phosphatase	
		(mg/dl)	%	IU/l	%*
Basal Diet	——	$97.67^{bc} \pm 2.91$	100.00	$111.35^{b} \pm 5.74$	100.00
Basal Diet (-Zn)	——	$89.33^{cde} \pm 1.45$	91.47	$133.70^{a} \pm 3.12$	120.07
Flat bread	——	$86.67^{de} \pm 3.33$	88.74	$70.85^{c} \pm 7.65$	63.63
	Soy bean flour[1]	$112.50^{a} \pm 3.18$	115.19	$37.00^{d} \pm 0.09$	33.23
	$ZnSO_4$ [2]	$82.33^{e} \pm 4.10$	84.30	$112.05^{b} \pm 7.48$	100.63
	ZnO[3]	$93.00^{bcd} \pm 3.06$	95.22	$64.95^{c} \pm 8.23$	58.33
	Soy bean flour + $ZnSO_4$[4]	$112.75^{a} \pm 1.59$	115.44	$39.40^{d} \pm 2.54$	35.38
	Soy bean flour + ZnO[5]	$100.33^{b} \pm 4.26$	102.73	$47.60^{d} \pm 4.33$	42.75
LSD (p<0.05)		9.391		16.761	

[a,b,c,d,e] Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at *P* < 0.05 percentage relative to control is reported in parameters.

[*] Relative to basal diet group.

Effect of flat bread supplemented with different sources of zinc on serum hormones 3, 5, ´3 triiodothyronine (T_3) and tetraiodothyronine (T_4) in hypozincemic rats:

Table (4) showed the effect of flat bread supplemented with different sources of zinc on T_3 and T_4 hormones. Non significant difference in serum T_3 was found in all groups when compared with basal diet, except rats fed on flat bread supplemented with ZnO or soy flour + ZnO. So, it can be say that the effect of ZnO is more than that $ZnSO_4$ on T_3 level. On the other hand, the lowest value of T_3 was found in rats fed on basal diet free Zn. Concerning T_4 level, it was significantly increased in all groups fed on flat bread or flat bread supplemented with different sources of zinc (except that supplemented with soybean flour only) compared with basal diet and basal diet free Zn. Generally, it can be concluded that zinc supplementation increased serum levels of T_3, T_4 in rats due to zinc deficiency impairs the metabolism of thyroid hormone. These results are in agreement and confirmed with results obtained by Kralik *et al.* (1996).

Table (4): Effect of feeding flat bread supplemented with different sources of zinc on serum 3, 5, ´3 triiodothyronine (T_3) and tetraiodothyronine (T_4) in hypozincemic rats

Diet	Supplements	3, 5, /3 triiodothyronine (T_3)		Tetraiodothyronine (T_4)	
		(ng/dL)	%	(µg/dl)	%
Basal Diet	___	$51.30^{bc} \pm 4.10$	100.00	$2.84^{d} \pm 0.10$	100.00
Basal Diet (- Zn)	___	$35.00^{c} \pm 2.89$	68.23	$1.93^{e} \pm 0.14$	68.08
Flat bread	___	$68.70^{b} \pm 5.95$	133.92	$4.31^{b} \pm 0.23$	151.85
	Soy bean flour[1]	$56.75^{b} \pm 0.43$	110.62	$3.25^{cd} \pm 0.32$	114.64
	$ZnSO_4$ [2]	$59.55^{b} \pm 4.94$	116.08	$3.61^{c} \pm 0.18$	127.16
	ZnO[3]	$112.00^{a} \pm 10.97$	218.32	$4.20^{b} \pm 0.06$	148.15
	Soy bean flour + $ZnSO_4$[4]	$66.20^{b} \pm 2.94$	129.04	$5.12^{a} \pm 0.11$	180.42
	Soy bean flour + ZnO[5]	$101.05^{a} \pm 10.94$	196.98	$5.15^{a} \pm 0.14$	181.66
LSD (p<0.05)		19.364		0.532	

[a,b,c,d,e] **Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at *P* < 0.05 percentage relative to control is reported in parameters.**

[*] **Relative to basal diet group.**

Effect of feeding flat bread supplemented with different sources of zinc on serum testosterone in hypozincemic rats:

Effect of different sources of zinc on gonad in hypozincemic rats was studied by determining the levels of serum testosterone as show in Table (5). It was observed a significant increase of serum testosterone in the rats fed on flat bread supplemented with soy flour, ZnO and soy flour + ZnO compared with basal diet free Zn and wheat flour (100%). So, ZnO is more effective in serum testosterone level than $ZnSO_4$. On the other hand, mixing of chemical and natural sources of zinc decreased the level of serum testosterone compared with other treatments.

Table (5): Effect of feeding flat bread supplemented with different sources of zinc on serum testosterone in hypozincemic rats

Diet	Supplements	Serum testosterone	
		(ng/ml)	%
Basal Diet	___	$1.10^{c} \pm 0.29$	100.00
Basal Diet (- Zn)	___	$0.32^{de} \pm 0.02$	28.79
Flat bread	___	$0.22^{e} \pm 0.02$	19.70
	Soy bean flour[1]	$1.80^{a} \pm 0.29$	163.64
	$ZnSO_4$ [2]	$0.45^{d} \pm 0.03$	40.91
	ZnO[3]	$1.50^{b} \pm 0.46$	136.36
	Soy bean flour + $ZnSO_4$[4]	$0.50^{d} \pm 0.12$	45.45
	Soy bean flour + ZnO[5]	$1.00^{c} \pm 0.29$	90.91
LSD (p<0.05)		0.199	

[a,b,c,d,e] **Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at *P* < 0.05 percentage relative to control is reported in parameters.**

[*] **Relative to basal diet group.**

It could be concluded that zinc deficiency decreased level of serum testosterone but zinc supplementation increased testosterone secretion in hypozincemic rats and it reached to the normal level as similar as rats fed on basal diet.

These results are in agreement with the results of Prasad *et al.* (1996).

Effect of feeding flat bread supplemented with different sources of zinc on zinc level in serum and tibia in hypozincemic rats:

The effect of feeding flat bread supplemented with various zinc sources and their mixtures on the level of zinc in serum and tibia was shown in Table (6). The results indicated that zinc in serum was significantly increased due to zinc supplementation with different sources (natural or chemical or their mixture) compared with basal diet free Zn and wheat flour (100%). Highly significant increase was found in serum zinc due to feeding flat bread containing 20% soy flour + ZnO and 20% soy flour + $ZnSO_4$ (3.74 and 3.41 ppm, respectively).

Table (6): Effect of feeding flat bread supplemented with different sources of zinc on zinc level in serum and tibia in hypozincemic rats

Diet	Supplements	Zinc in tibia		Zinc in serum	
		ppm	%	ppm	%*
Basal Diet	——	$312.17^{de} \pm 2.62$	100.00	$2.68^{b} \pm 0.09$	100.00
Basal Diet (- Zn)	——	$291.00^{e} \pm 8.09$	93.22	$1.790^{c} \pm 0.05$	66.79
Flat bread	——	$244.46^{f} \pm 8.69$	78.31	$1.58^{c} \pm 0.14$	58.96
	Soy bean flour[1]	$324.07^{cd} \pm 3.47$	103.81	$2.84^{b} \pm 0.05$	105.97
	$ZnSO_4$ [2]	$404.05^{a} \pm 1.59$	129.43	$2.68^{b} \pm 0.12$	100.00
	ZnO[3]	$364.88^{b} \pm 19.16$	116.89	$2.82^{b} \pm 0.21$	105.22
	Soy bean flour + $ZnSO_4$[4]	$335.00^{cd} \pm 4.19$	107.31	$3.410^{ab} \pm 0.38$	127.24
	Soy bean flour + ZnO[5]	$344.88^{bc} \pm 10.53$	110.48	$3.74^{a} \pm 0.53$	139.55
LSD (p<0.05)		27.15		0.83	

[a,b,c,d,e] **Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at *P* < 0.05 percentage relative to control is reported in parameters.**

[*] **Relative to basal diet group.**

It can be concluded that supplementation increased zinc level in serum which plays an important role in the immunsystem, growth, metabolism, decrease of total cholesterol, triglycerides and increase the testosterone level and regulation of T_3 and T_4 (El-Hendy *et al.,* 2001).

Concerning zinc level in tibia, it could be noticed that there is a significant increase of zinc content of tibia in rats fed flat bread supplemented with natural and chemical sources of zinc compared with that fed diet (free Zn) and wheat flour (100%). The highest value of zinc m tibia was recorded by feeding flat bread containing $ZnSO_4$ followed by ZnO.

The increase of zinc level in tibia followed by increase in weight of tibia indicates that zinc plays an important role in the development at the skeletal system (Nishi, 1996).

CONCLUSION

From this study, it can be concluded that supplementation of flat bread with natural and chemical sources of zinc increased body weight and testosterone level, and regulate the thyroid hormones.

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دراسات بيولوجية على الخبز المدعم بمصادر مختلفة من الزنك

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** قسم بحوث تكنولوجيا المحاصيل – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية**

تم تغذية الفئران المصابة بنقص الزنك على الخبز المدعم بالمصادر المختلفة للزنك سواء مصادر طبيعية (٢٠٪ دقيق فول صويا) أو مصادر كيماوية (أكسيد زنك – كبريتات زنك بنسبة ٨ ملجم / ١٠٠ جم دقيق) ومقارنتها بالفئران التي تغذت على خبز غير مدعم (١٠٠٪ دقيق قمح ٧٢٪). أجريت التحاليل البيولوجية المختلفة على الفئران مثل: وزن الفئران، وزن الأعضاء، مستوى الجلوكوز، الألكالين فوسفاتيز، هرمونات الغدة الدرقية (T_3, T_4)، هرمون الذكورة وكذلك نسبة الزنك في مصل الدم وفى عظمة الساق الكبرى. وقد أوضحت النتائج أن التغذية على الخبز المدعم بمصادر مختلفة من الزنك أدت إلى زيادة في وزن الفئران وكذلك وزن الأعضاء. وقد لوحظ أن التدعيم بمصادر الزنك الكيماوية أدت إلى انخفاض مستوى الجلوكوز بالدم مقارنة بالمصادر الطبيعية. كما أدى التدعيم بالزنك إلى زيادة في هرمونات الغدة الدرقية وهرمون الذكورة وكذلك نسبة الزنك في مصل الدم و عظمة الساق الكبرى.

Table (1): Effect of feeding flat bread supplemented with different sources of zinc on body weight and feed intake of rats.

Diet	Supplements	Initial weight	Final weight (4 weeks)	B. wt. gain		Daily b. wt. increase	Daily feed intake	Feed intake (4 week)	F.E.R
		(g)	(g)	(g)	%	(g)	(g)	(g)	
Basal Diet	——	63.40[a] ±5.07	155.50[a] ±9.60	92.10	145.27	3.29	13.30	372.40	26
Basal Diet (- Zn)	——	63.60 [a] ±5.26	72.67[c] ±4.71	9.07	72.96	0.32	11.40	319.20	16
Flat bread	——	63.40 [a] ±5.85	125.25[b] ±4.87	61.85	97.56	2.21	12.20	341.60	18
	Soy bean flour[1]	63.40 [a] ±8.63	168.75[a] ±12.33	105.35	166.17	3.76	13.50	378.00	28
	$ZnSO_4$ [2]	63.40 [a] ±6.29	151.50[ab] ±9.84	88.10	138.96	3.15	12.99	363.72	24
	ZnO[3]	63.60 [a] ±8.54	147.25[ab] ±9.45	83.65	131.53	2.99	12.70	355.60	24
	Soy bean flour + $ZnSO_4$[4]	63.80 [a] ±6.34	162.75[a] ±11.37	98.95	155.09	3.53	14.00	392.00	26
	Soy bean flour + ZnO[5]	63.60 [a] ±7.35	156.75[a] ±6.51	93.15	146.46	3.33	12.60	352.8	28
LSD (p<0.05)		19.60	26.27						

[1] 20% soybean flour, [2] 80mg Zn/Kg diet as $ZnSO_4$, [3] 80mg Zn/Kg diet as ZnO, [4] 20% soybean flour + 80mg Zn/Kg diet as $ZnSO_4$ and [5] 20% soybean flour + 80mg Zn/Kg diet as ZnO

[a,b,c,d,e] Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at P < 0.05 percentage relative to control is reported in parameters.

- Each value represents the mean of 5 rats (Mean ± SE). B. wt. Body weight F. F. R. = Feed efficiency ratio (B. W. gain/ feed intake) ×100

Table (2): Effect of feeding flat bread supplemented with different sources of zinc on organs weight in rats

Diet	Supplements	Final body weight	Liver		Testes		Brain		Kidney		Spleen		Heart		Tibia	
		(g)	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%
Basal Diet	——	155.50	6.65^{ab} ±0.57	4.28	1.98^{a} ±0.10	1.27	1.38^{ab} ±0.08	0.89	0.97^{ab} ±0.11	0.62	0.42^{ab} ±0.10	0.27	0.50^{a} ±0.06	0.32	0.38^{ab} ±0.02	0.24
Basal Diet (- Zn)	——	72.67	3.28^{c} ±0.45	4.51	0.62^{b} ±0.33	0.85	1.31^{b} ±0.01	1.80	0.58^{c} ±0.08	0.80	0.37^{b} ±0.10	0.51	0.36^{b} ±0.05	0.50	0.19^{c} ±0.02	0.26
Flat bread	——	125.25	5.29^{b} ±0.68	4.22	1.73^{a} ±0.08	1.38	1.33^{b} ±0.06	1.06	0.79^{b} ±0.05	0.63	0.25^{c} ±0.07	0.20	0.49^{a} ±0.03	0.39	0.34^{b} ±0.01	0.27
	Soy bean flour[1]	168.75	5.91^{ab} ±0.32	3.50	1.95^{a} ±0.18	1.16	1.45^{ab} ±0.04	0.86	1.09^{a} ±0.03	0.65	0.53^{ab} ±0.18	0.31	0.58^{a} ±0.03	0.34	0.44^{a} ±0.03	0.26
	$ZnSO_4$ [2]	151.50	5.95^{ab} ±0.36	3.93	2.09^{a} ±0.09	1.38	1.43^{ab} ±0.03	0.94	1.04^{a} ±0.03	0.69	0.33^{b} ±0.04	0.22	0.58^{a} ±0.03	0.38	0.42^{a} ±0.01	0.28
	ZnO[3]	162.75	5.33^{b} ±0.38	3.27	2.02^{a} ±0.02	1.24	1.44^{ab} ±0.01	0.88	1.09^{a} ±0.08	0.67	0.61^{a} ±0.22	0.37	0.56^{a} ±0.04	0.34	0.43^{a} ±0.02	0.26
	Soy bean flour + $ZnSO_4$[4]	147.25	6.99^{a} ±0.48	4.75	1.71^{a} ±0.20	1.16	1.50^{a} ±0.03	1.02	1.04^{a} ±0.07	0.71	0.52^{ab} ±0.13	0.35	0.55^{a} ±0.03	0.37	0.40^{ab} ±0.01	0.27
	Soy bean flour + ZnO[5]	156.75	6.70^{ab} ±0.63	4.27	1.86^{a} ±0.14	1.19	1.41^{ab} ±0.07	0.90	1.15^{a} ±0.03	0.73	0.66^{a} ±0.05	0.42	0.58^{a} ±0.02	0.37	0.42^{a} ±0.03	0.27
LSD (p<0.05)			1.46		0.41		0.14		0.19		0.37		0.12		0.04	

[a,b,c,d,e] **Values are means of three replicates ± SE, number in the same column followed by the same latter are not significant different at *P* < 0.05 percentage relative to control is reported in parameters.**