

Protective Effects of Vitamin E, Zinc and Selenium Supplementation on Growth Performance and Some Biochemical Parameters in Growing Rabbits Exposed to Cadmium

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ABSTRACT

The current study was carried out at Kafr El-Sheikh Governorate during 2016 to investigate the effect of using three water quality; Nile water (Abou-Omar, W1), mixed Nile and polluted drainage water (El-Bana, W2) and polluted drainage water (Kafr Dokhmeis, W3) in irrigation of agricultural land on accumulation of Cd in clay soil and berseem grown thereon and subsequently in growth performance of rabbits fed on diets contaminated with cadmium at same level of cadmium in this berseem. The role of Selenium (Se), Vitamin E (Vit. E), or Zinc (Zn) as antioxidants and as detoxifying against cadmium chloride (CdCl₂) was studied in 120 growing APRI rabbits. Rabbits were classified into six groups. Rabbits of the treatment 1 (T1) served as diet as a control diet contained 0.10 mg cadmium (Cd) on DM basis. Treatment 2 (T2): Fed contaminated diet to provide final level of 0.96 mg Cd, Treatment (3): Fed diet supplemented with cadmium chloride plus vitamin E (150 mg/kg diet). Treatment 4 (T4): Fed diet supplemented with cadmium chloride plus Se (0.2 mg/kg diet). Treatment 5 (T5): Fed diet supplemented with cadmium chloride plus Zn (100 mg/kg diet) and Treatment 6 (T6): Fed diet supplemented with cadmium chloride plus 150 mg vit E plus 0.2 mg Se plus 100 mg Zn /kg diet). The results showed that polluted drainage water (W3) recorded the highest concentrations of Cd followed by W2, while W1 had the lowest concentrations. Moreover, berseem samples grown in soil irrigated with W3 showed the highest Cd content followed by berseem grown in soil irrigated with W2, while berseem grown in soil irrigated with W1 had the lowest contents. Cadmium chloride treated group showed significantly a decrease in growth performance and nutrient digestibility while supplementation with vit. E, Se or Zn alone or in combination to the contaminated diet significantly improved all these growth parameters. In the contaminated diet with Cd (T2), many large disturbance occurred in all measured blood constituents, while supplementing vit E, Se or Zn alone or in combination to the same diet, significantly improved these blood constituents. Moreover, vitamin E, Se and Zn combination showed more efficacy than vit. E, Se or Zn alone.

Keywords: antioxidants, cadmium, detoxification, growth, blood, rabbits

INTRODUCTION

Cadmium is the most dangerous environmental pollution as it's also unique due to its toxicity and its little excretion from the body (Kour *et al.*, 2014 and Olgun, 2015). On the other hand, we can find it in nature, soils, air and water system. In fact it is produced by the burning fossils fuels and metal refining (Chen *et al.*, 2008 and Kour *et al.*, 2014). You can find a lot of cadmium in soils because of agriculture activities (e.g. sewage sludge, phosphate fertilizers, and pesticides) all of them contain a lot of cadmium (Abdo and Abdulla, 2013). So it is considered as one of the carcinogen (IARC, International Agency For Research on Cancer, 1993) and it is half-life in the human kidney is 18 to 33 years (Gail *et al.*, 1982). When animals or human absorb it its excreted in little amounts. So the body does great effort to stop its entry to the human food chain. Maximum tolerable levels of dietary cadmium for avian species were set at 0.5 mg/kg diet (El-Deek *et al.*, 2010). There are a lot of adverse effects of toxic cadmium as kidney damage, anemia, behavioral responses, energy metabolism, adrenal hypertrophy and cardiac. Some studies see that the cadmium no effect on the bone, but other studies say that cadmium causes renal dysfunction which effect on the skeletal (Whelton *et al.*, 1997a & b). The inactivation of enzymes and functional proteins cause the general toxic effect of heavy metal by directly binding to them (Tsuiji *et al.*, 2002).

Stohs and Bagchi (1995) reported that oxidative damage partially by formation of reactive oxygen species (ROS). Oxidative stress developing with the production of can lead to the development of many pathological changes (Morakinyo *et al.* 2012). For example, Cd has been

attributed to the formation of reactive oxygen species (Bharathi *et al.* 2012). Superoxide dismutase, catalase and glutathione peroxidase (antioxidant enzymes) protect cellular homeostasis from oxidative damage by reactive oxygen species generated through the reduction of molecular oxygen (Sanz *et al.* 2002). Antioxidants decrease ROS-induced damage (El-Demerdash, 2004). Se is an essential trace element for humans and animals, which prevents the cells against oxidative damage by the expression of selenoprotein genes and through anti-inflammatory mechanisms (Said *et al.* 2014). It is an integral component of the cytosolic enzyme GPx and facilitates the action of Vit. E in decreasing peroxy radicals (Kaneko 1989). Se has protection effect on many heavy metals (Diplock *et al.* 1986). Vit. E (α -tocopherol) is a naturally occurring, potent lipid-soluble, chain-breaking antioxidant. It protects cellular membranes and lipoprotein surfaces from lipid peroxidation (Al-Othman *et al.* 2011). Its protective role has been reported against the heavy metal toxicity in experimental animals (Agarwal *et al.* 2010). Synergistic effect of antioxidants such as vit. E, Se, Zn and is the most powerful in decreasing storage and toxicity of reactive oxygen species (Aslam *et al.* 2010). Antioxidant addition has a protective role in metal toxicity. Therefore, the current study aims to investigate the effect of environmental pollution, represented in soil, irrigation water containing heavy metals, on the growth and production of Egyptian clover; a common fodder plant in Egypt. It also investigates the potential accumulation of cadmium in the berseem, which may cause health risk for animals and humans through the food chain. Also, the current work aimed to study the effect of Vit E, Se or Zn on growth performance, some blood constituents, lipid peroxidation (MAD) and antioxidant enzyme activities (

CAT and GSH) of serum of growing rabbits fed diets contaminated by Cd.

MATERIALS AND METHODS

Detection of Cd concentration in berseem, clay soil and water collected from different areas

The present study was carried out at Kafr El-Sheikh Governate during 2016 to investigate the effect of using three water quality sources; Nile water (Abou- Omar, W1), mixed Nile and polluted drainage water (El-Bana, W2) and polluted drainage water (Kafr Dokhmeis, W3) in irrigation of agricultural land on accumulation of cadmium in the soil and berseem grown thereon. Representing samples of water, soil and berseem were collected from the studied areas for cadmium determination. Water and soil clay samples were collected and analyzed by the same way using aqua regia method described by Cottenie *et al.* (1982). Berseem samples was prepared for cadmium determination according to AOAC (1990).

Animals and feeding system:

The current work was carried out at the Agriculture Research Center Rabbits, Animal Production Research Institute, Farm of Sakha Station, Egypt. At total of 120 growing APRI rabbits were randomly distributed into six groups of 20 rabbits each (10 females + 10 males) at 5 weeks of age were used in this study. Rabbits were housed in individual cages (60 x 50 x 35 cm) in a well ventilated

(with open windows and ventilating fans building). Fresh water was automatically available all the time by stainless steel nipples. All rabbits were raised under the same managerial, hygienic and environmental conditions.

The basal experimental diet was formulated and pelleted to cover the nutrient requirements of rabbits according to the available nutrients as shown in Table 1. The experimental groups were as follow:

Treatment 1 (control): fed basal diet containing 0.1mg cadmium (Cd) on DM basis.

Treatment 2: fed basal diet contaminated with cadmium chloride (1.08 mg/kg diet) to provide Cd content of 0.96 mg (as indicated from Cd content of berseem from contaminated areas at Kafr El-Sheikh Governorate) (contaminated diet).

Treatment 3: fed contaminated dietplus 150mg vit. E /Kg diet.

Treatment4: fed contaminated dietplus 0.2 mg organic selenium / Kg diet.

Treatment5: fed contaminated dietplus 100 mg organic zinc / kg diet.

Treatment6: fed contaminated dietplus 150mg vit. E - 0.2 mg Se – 100mg Zn /Kg diet.

Source of different additives:

Heavy Metals: Cadmium was supplied as cadmium chloride (CdCl₂; 99% purity)

Table 1. Chemical constituents of the basal diet.

Ingredients	%	Chemical composition (% as DM):	%
Barley grain	24.60	DM	85.81
clover hay	30.05	CP	17.36
Soybean meal (44% CP)	17.50	OM	91.42
Wheat bran	21.50	CF	12.37
Molasses	3.00	EE	2.230
Limestone	0.95	Digestible energy(DE, kcal/kg) ⁽²⁾	2412
Salt	0.30	Methionine ⁽²⁾	0.454
Di-calcium phosphate	1.60	Lysine ⁽²⁾	0.862
Mineral-vitamin premix ⁽¹⁾	0.30		
DL-Methionine	0.20		
Total	100		

(1) Vitamins and Minerals per kilogram : Vit. A, 150,000 UI; Vit. B1, 10 mg;; Vit. K3, 21mg ; Vit. E, 100 mg; Vit. B6, 15mg; Vit. B2, 40mg; Pantothenic acid, 100 mg; Niacin, 200 mg; Vit. B12, 0.1mg; Biotin, 0.5mg; Choline chloride, 5000 mg; Mn, 600 mg;Folic acid, 10 mg; Cu, 50 mg; Co, 2 mg; Fe, 0.3mg; Zn, 450mg and.Se, 1mg.

Calculated according to NRC (1980).

Antioxidants:

¹⁻ Vitamin E (α - tocopherol acetate from natural source; each gram contains a minimum of 889 mg of D-tocopherolacetate, equivalent to 1100 IU), produced by Hoffmann-LaRoche).

2- Organic selenium was SelPlexTM in the form of selenium yeast (Alltech Inc.) contained 1000 ppm organic selenium and produced by the fermentation of yeast (*Saccharomyces cerevisiae*) in a high organic selenium medium.

3- Zinc-Methionine complex was obtained from Ibox company , Egypt and the purity of Zinc-Methionine complex was 98%.

Rabbits were fed *ad libitum* throughout the experimental period (8weeks)and daily feed consumption was recorded. All rabbits were weighed individually at the beginning of the experiment and biweekly thereafter.

Average daily weight gain and feed conversion were calculated.

Blood samples were collected during sacrificing rabbits at of the experiment in dry tubes. Samples of serum were obtained and stored at -20°C to be used for biochemical analysis. All blood serum (serum total protein, albumin; while globulin was obtained by subtracting the concentration of albumin from that of total protein; urea, creatinine, total glucose, cholesterol, aspartate amino transferase (AST), alanine amino transferase (ALT), and Alkaline phosphatases (ALP) were estimated by commercial kits.

Total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in serum were also measured using commercial kits obtained from Biodiagnostic, Giza, Egypt

Digestibility trial:

A digestibility trail was estimate to determine the digestibility coefficient of the six diets used. Three rabbits from each group housed individually in metabolic cages separation of faeces and urine. Digestibility trial lasted 15 days (10 days as a preliminary period and 5 days as collection period). Samples from both feed offered and faeces of each rabbit were taken daily during the collection period for chemical analysis that carried out according to AOAC (1995). The total digestible nutrients (TDN) and digestible crude protein (DCP) were calculated according to by Perez *et al.*, (1995).

Statistical analysis:

The data obtained in this study were analyzed by using the general linear GLM procedure of SAS program (SAS, Institute, Inc., 2002). Significant differences among means were detected by the method of Duncan (1955).

RESULTS AND DISCUSSION

The concentration of cadmium in different types of water is present in Table (2). There were differences among the water types in the cadmium concentration, where W3 recorded the highest concentration followed by W2, while W1 had the lowest concentrations. The concentration of Cd in W3 was higher than the normal level of cadmium recommended in irrigation water being 0.01 mg/l for Cd (FAO, 1976 and WHO, 1993). Zein *et al.* (2002a,b) found that heavy metals content were higher in drainage water than Nile water.

Data in Table (2) showed the contents of cadmium in clay soil and berseem as affected by their contents in

water. There was significant differences on cadmium contents in samples of berseem and soil from the different locations, which reflects similar trend to their contents in water. Soil and berseem samples irrigated with W3 showed the highest cadmium content followed by those irrigated with W2, while soil and berseem irrigated with W1 had the lowest contents. Similar these results agree with Rusan *et al.* (2006) they reported that plant Cu, Pb, Mn and Cd increased with waste water irrigation. Heavy metals such as Cu, Pb and Cd are potential bioaccumulative toxicants for the dairy production system as soils tend to act as long term sinks for these metals (Alloway, 1995) via sorption onto metal oxides, particularly manganese and iron oxides (Brown and Parks, 2001). Abd El-Hady (2007) reported that much higher Mn, Cu, Ni, Pb and Cd in plant grown in drainage water irrigated lands compared to plant grown in Nile irrigated lands. The concentration of Cd in Berseem samples irrigated with W3 were higher than the maximum tolerable level of this element by rabbits being 0.5 ppm (NRC, 1980). Dheri *et al.* (2007) who found that sewage water contain high amount of cadmium. Therefore, it is possible that cadmium had been accumulated in the soil from which the forages were harvested. Due to high levels of cadmium in sewage water compare to that in canal water, it is expected that forages irrigated with canal water would have the lowest cadmium level and those irrigated with sewage water would have the highest cadmium level. Zein *et al.* (2002a) reported that the contents of heavy metals in wheat straw were greater when poor water quality was used for irrigation than that of the mixed or good water quality.

Table 2. Cadmium concentration in different type of water, clay soil and berseem were collected from the studied areas

Type	Cadmium concentration			SEM	p-value
	W1	W2	W3		
Water, (ppm/l)	0.003 ^c	0.010 ^b	0.162 ^a	0.025	0.0001
clay soil, (ppm on DM basis)	0.10 ^b	0.18 ^b	0.42 ^a	0.031	0.003
berseem, (ppm on DM basis)	0.20 ^c	0.41 ^b	0.96 ^a	0.073	0.0001

SEM = Standard error of means. W1: Nile water (Abou-Omar), W2: mixed Nile and polluted drainage water (El-Bana) and W3: polluted drainage water (KafriDokhmeis).

Growth parameters:

Average live body weight, daily gain, feed consumption , FCR, relative growth rate and the growth performance index (PI) of growing rabbits give contaminated diets with cadmium during different period of the experimental period are shown in Table (3). There were differences in average live body weight, daily gain, feed consumption, FCR and relative growth rate among growing rabbits in the experimental groups were not significant during the first interval (5-9 weeks of age). However, average live body weight, daily gain, feed consumption and FCR during the intervals from 9 to 13 weeks of age and the overall mean of average live body weight, daily gain, feed intake , FCR and PI (%) from 5 to 13 weeks of age were significantly lower in rabbits exposed to Cd (T2) than those fed the other groups. Average live body weight, daily gain, feed consumption and FCR of rabbits in group fed contaminated diet with Cd decreased by 27.63, 38.54, 14.39 and 39.34% compared

with the control group (T1), respectively. These results revealed an accumulative effect of the diets contaminated with Cd. The reduced weight gain of rabbits found in this study is agreement with (Horiguchi *et al.*, 1996; Abd-Elgawad *et al.* (1999) and Ibrahim *et al.* (2000). So, the reduction in growth performance could have been due to the reduce in feed consumption , or due to the overall increase in degradation of proteins and lipids as a result of adverse effects of Cd (Erdogan *et al.*, 2005). However, vitamin E, Se or Zn either alone or in combination and supplies, the growth performance became significantly higher (p < 0.005) than in rabbits fed diet contaminated with cadmium. These results similar with results reported by Messaoudi *et al.* (2009), who found that Se increased the body weight of Wistar rats fed diet contaminated with cadmium. Also, Layachi and Kechrid (2012) found that the supplementation of vit. E and/or vit. C to animals diets contaminated with cadmium improved body weight.

Table 3. Growth performance traits of APRI rabbits fed diets containing cadmium supplemented with vit. E, Se and Zn from 5 to 13 wks of age.

Traits	Experimental diets*						SEM	P-value
	T1	T2	T3	T4	T5	T6		
No of rabbits at 5 wk	20	20	20	20	20	20	--	--
Final number at 13 wk	19	17	18	18	18	19	--	--
Initial body weight (g)	650	648	648.3	653.0	648.6	650.0	5.30	1.00
Final body weight (g)	2280 ^a	1650 ^c	2000 ^b	1970 ^b	1997 ^b	2100 ^b	17.11	0.0001
Daily weight gain (g):								
5-9 weeks	27.32	24.00	26.36	26.26	26.12	26.76	0.13	0.063
9-13weeks	30.89 ^a	11.78 ^d	21.90 ^{bc}	20.77 ^c	22.02 ^{bc}	25.05 ^b	0.660	0.0001
5-13 weeks	29.11 ^a	17.89 ^d	24.14 ^{bc}	23.52 ^c	24.07 ^{bc}	25.89 ^b	0.311	0.0001
Feed intake (g/d):								
5-9 weeks	81.00	79.95	80.96	80.67	80.53	80.93	0.281	.925
9-13weeks	132.35 ^a	100.67 ^c	126.07 ^b	125.46 ^b	126.11 ^b	128.21 ^b	0.361	0.0001
5-13 weeks	106.67 ^a	91.32 ^c	103.52 ^b	103.07 ^b	103.32 ^b	104.57 ^b	1.234	0.0001
Feed conversion ratio :								
5-9 weeks	2.96	3.33	3.07	3.07	3.09	3.03	0.037	0.078
9-13weeks	4.29 ^c	8.54 ^a	5.79 ^b	6.05 ^b	5.73 ^b	5.20 ^{bc}	0.349	0.0001
5-13 weeks	3.66 ^c	5.10 ^a	4.30 ^b	4.39 ^b	4.29 ^b	4.05 ^b	0.112	0.0001
Relative growth rate :								
5-9 weeks	74.11	68.28	77.53	72.01	72.08	73.24	0.766	0.362
9-13weeks	46.78 ^a	22.22 ^d	36.17 ^{bc}	34.63 ^c	36.54 ^{bc}	40.00 ^b	1.853	0.0001
5-13 weeks	111.25 ^a	87.20 ^c	101.98 ^b	100.37 ^b	101.92 ^b	105.49 ^b	1.845	0.0001
Performance index (%)	62.25 ^a	32.35 ^c	46.73 ^b	45.01 ^b	46.52 ^b	52.11 ^b	2.305	0.0001
Mortality (%)	5 ^c	15 ^a	10 ^b	10 ^b	10 ^b	5 ^c	0.022	0.002

T1 (control): 0.28 ppm Cd , T2: Cd-diet, T3: Cd-diet plus vit E , T4: Cd-diet plus Se, T5: Cd-diet plus Zn and T6: Cd-dietplusvit. E plus Se plus Zn ;SEM = Standard error of means,

Mortality rate:

Mortality rate of contaminated groups with the high levels of Cd (T2) was higher than those fed the control and the other treatments. Similar results obtained by Swaileh *et al.* (2002) they found that cadmium proved to cause the highest rate of mortality. Treatments with vitamin E, Se, Zn alone and their combination decreased mortality rate.

Digestibility coefficients:

Digestibility coefficients of nutrients in different experimental groups are shown in Table (4). The present results show that digestibility coefficients of CP, EE and NFE were significantly decreased in group fed diet contaminated with Cd as compared to the control group. The corresponding reduction in nutrient digestibility coefficients with increasing Cd contamination may be related to the toxic effect of high dose on microorganisms in gastrointestinal tract (GIT) of rabbits, especially in the caecum, which in turn causes an adverse effect on caecal

fermentation. Regarding the nutritive values, we notice that TDN was affected significantly ($P < 0.05$) by the highest dose of contamination with Cd (0.96 mg, T2). This was associated with the significant reduction in digestibility coefficients of CF in this treatment compared to the other treatments. However, nutritive value such as DCP was significantly lower in T2 than the other groups. Such trends were attributed to CP digestion in the experimental treatments. Similar results were obtained by Bersenyi *et al.* (1999) who found that Cd intake resulted in a considerable reduce in nutrients digestibility. Ibrahim *et al.* (2000) found that Cd contamination decreased digestibility coefficients by growing rabbit. Fekete *et al.* (2001) reported that both beet and potato root of high cadmium and Pb contents had the significantly lowest digestibility of nutrients. However, groups with vit. E, Se, Zn alone and a combination improved digestibility coefficients by growing rabbits fed diet contaminated with cadmium.

Table 4. Apparent digestibility for rabbits fed the experimental diets .

Parameters	Experimental diets*						SEM	p-value
	T1	T2	T3	T4	T5	T6		
Nutrients digestibility coefficients %:								
CP	74.83 ^a	65.41 ^c	73.60 ^b	73.00 ^b	73.45 ^b	74.90 ^a	0.801	0.0001
CF	24.5 ^a	21.60 ^c	23.10 ^b	24.0 ^a	23.65 ^b	24.10 ^a	0.247	0.0001
EE	81.20 ^a	74.55 ^d	79.20 ^{bc}	78.90 ^c	79.00 ^c	80.05 ^b	0.510	0.0001
NFE	73.42 ^a	68.17 ^c	70.52 ^b	71.48 ^b	70.96 ^b	71.30 ^b	0.173	0.0001
Nutritive values %:								
TDN	60.28 ^a	55.08 ^c	58.20 ^b	58.72 ^b	58.48 ^b	59.02 ^b	0.394	0.0001
DCP	13.00 ^a	11.36 ^c	12.77 ^b	12.67 ^b	12.75 ^b	13.00 ^a	0.139	0.0001

T1 (control): 0.28 ppm Cd , T2: Cd-diet, T3: Cd-diet plus vit E , T4: Cd-diet plus Se, T5: Cd-diet plus Zn and T6: Cd-dietplusvit. E plus Se plus Zn., TDN: totaldigestible nutrient , DCP: digestible crude protein.

Blood biochemical parameters:

Serum of animals exposed to contaminated diet showed significantly increased of serum AST, ALT , ALP, glucose, creatinine and urea levels compared with the control group (T1). In addition, the level of T. protein ,

albumin and cholesterol in serum were decreased ($p < 0.05$). While , supplementation of vit. E, Se or Zn alone or with Cd caused on increase in T. protein , albumin and globulin (Table 5). In addition, the activities of enzymes (AST, ALT and ALP) in addition to the levels of glucose, creatinine and urea were significantly reduced in rabbit

groups fed diets with feed additives (vit. E, Se or Zn either alone or in combination). While, vit. E, Se and Zn combination showed more efficacy than vit. E, Se or Zn individually (Table 5). These results agree with Layachi and Kechrid (2012). Cadmium can reduce T. protein and albumin in serum of rabbit by changes in protein synthesis and/or metabolism and induce membrane damage (Dostal *et al.*, 1989; Das Dasgupta, 2000 Yousuf, 2002 and Uyanik *et al.*, 2001). However, the enzymes of AST, ALT and ALP were increased, compared to the control group. It

could be related to the hepatic damage resulting in increased releasing and leakage out of these enzymes from the liver cytosol into the blood stream which gives an indication on the hepatotoxic effect of this metal (Pari and Murugavel, 2005). The addition of vit.E, Se or Zn had prevented liver function from the adverse effects of cadmium as indicated by the significant restoration of serum total protein, glucose, albumin, ALT, AST, and ALP.

Table 5. Some biochemical constituents in blood of growing APRI rabbits fed diets containing cadmium supplemented with vit. E, Se and Zn at 13 wks of age.

Items	Experimental diets						SEM	p-value
	T1	T2	T3	T4	T5	T6		
T. protein (mg/dl)	6.53 ^a	5.45 ^c	5.93 ^{bc}	6.06 ^{ab}	5.81 ^{bc}	6.31 ^{ab}	0.099	0.006
Albumin (mg/dl)	3.78	3.15	3.26	3.32	3.49	3.43	0.080	0.304
Globulin (mg/dl)	2.75 ^a	2.30 ^b	2.66 ^{ab}	2.73 ^a	2.32 ^b	2.88 ^a	0.066	0.015
Cholesterol (mg/dl)	54.33 ^b	65.03 ^a	56.66 ^b	56.16 ^b	55.16 ^b	55.16 ^b	1.072	0.010
AST (IU/L)	51.63 ^b	77.05 ^a	60.06 ^b	60.91 ^b	57.33 ^b	52.99 ^b	2.320	0.002
ALT (IU/L)	14.16 ^b	18.97 ^a	16.02 ^b	14.45 ^b	14.76 ^b	14.76 ^b	0.467	0.002
ALP (IU/l)	50.16 ^d	73.26 ^a	58.58 ^b	58.13 ^{bc}	58.16 ^{bc}	53.60 ^{cd}	1.815	0.0001
Glucose (mg/dl)	77.06 ^d	130.50 ^a	96.43 ^b	87.83 ^c	90.06 ^c	80.86 ^d	4.307	0.0001
Creatinine (mg/dl)	0.89 ^c	1.85 ^a	1.18 ^b	1.13 ^b	1.27 ^b	1.00 ^{bc}	0.031	0.03
Urea (mg/dl)	14.06 ^b	27.93 ^a	13.96 ^b	12.50 ^b	15.06 ^b	13.93 ^b	0.885	0.025
Antioxidants status:								
TAC, nmol/ml	1.62 ^a	.983 ^b	1.42 ^a	1.54 ^a	1.46 ^a	1.43 ^a	0.057	0.003
GSH-PxU/g protein	1.44 ^a	.716 ^c	1.05 ^b	1.08 ^b	1.06 ^b	1.20 ^b	0.055	0.0001
MAD, nmol/ml	16.23 ^b	24.50 ^a	17.60 ^b	14.33 ^b	18.20 ^b	16.80 ^b	0.885	0.002

T1 (control): 0.28 ppm Cd T2: Cd-diet, T3: Cd-diet plus vitE , T4: Cd-diet plus Se, T5: Cd-diet plus Zn and T6: Cd-diet plus vit. E plus Se plus Zn. SEM = Standard error of means, TAC: Total antioxidant capacity, MAD: Malondialdehyde,

Cadmium chloride intoxicated rabbits also showed a high concentration of glucose. The high level in glucose is a joint result of heavy metal toxicity and is usually attacked to reduction of insulin releasing from Langerhans' islets (Dorner *et al.*,1973; Kechrid *et al.*, 2006) or with a block of glucose utilization by cells even in the presence of elevated levels of insulin (Sunderman *et al.*, 1976) or due to perturbation in glucagon secretion result in high glycogen breakdown and new supply of glucose production from protein sources (Massanyi *et al.*, 1995). However, there is an improvement of glucose level in blood for rabbits fed Cd supplemented with vit. E , Se or Zn alone or together. There are many possible explanations for the reduction in serum glucose. Addition of vitamin E, Se or Zn may improve insulin receptors in adipose tissue or muscle by enhancing membrane motility, secondary to increase glucose uptake by the diaphragm (Bierenbaum *et al.*, 1985).

Cadmium exposure produced a reduction in serum GSH-Px ,TAC while increase serum MAD compared with the control and the other groups (Table 5). Groups with vit. E, Se, Zn alone and a together improved these measurements. The reduction of GSH-Px and TAC level in animals exposed to cadmium may be as a result of oxidative stress, which has occurred of the adverse effects of cadmium. The reduction in antioxidant production due to the enhanced oxygen metabolites and the increased free radicals, which rised a reduce in the activity of the antioxidant defense system (Gstraunthaler *et al.*, 1983). Many explanations have been suggested to show the reduction of glutathione concentration in the adverse effects of heavy metals. Firstly, glutathione may be oxidized, due to the interaction with the free radicals, induced by nickel. Therefore, glutathione concentration could be consumed during nickel detoxification (Manna *et al.*, 2008)..

Secondly, the sulfhydryl group of cysteine moiety of glutathione has a high affinity of metals, forming thermodynamically stable mercaptide complexes with many metals. These complexes are inefficient which can be excreted via the bile (Aposhian, 1989). In addition, the reduction of GSH-Px activity in animals exposed to cadmium proposes that either there is an interaction between the accumulated free radicals and the active amino acids of this enzymes (Das *et al.*, 2001) or there is a direct binding of the metal to the active sites of the enzyme (Misra *et al.*, 1990). Supplementation of vit. E , Se or Zn alone or in combination caused significant improved glutathione and TAC levels while, MAD decreased when compared with T2. In the present study, glutathione peroxidase activity showed significant decrease in group exposed to cadmium. The decreased activity of the GSH-Px may result from a direct depletion of selenium by Cd or from insufficient incorporation of sulphur containing amino acids into GSH-Px because of competition with the Cd –metallothioneins synthesis (Baradaran.*et al.*, 2013). Moreover, Shaikh, *et al.* (1999) reported that, exposed to cadmium decreases the activities of many enzymes by inhibiting the protein synthesis or by binding to their sulfhydryl groups .

GSH-Px activity showed significant increase by cadmium with selenium-exposed rabbits. Similar observations were reported by Ognjanovic *et al.* (2008) who mentioned that treatment with selenium protect the adverse effects of cadmium on the activity of GSH-Px and prevents lipid peroxidation and oxidative damage induced by cadmium. This prevention involves the ability of selenium to improve the cadmium distribution in tissues and to stimulate bound of Cd-Se compounds to proteins which are similar to metallothioneins. Also, it has been suggested that Se addition elevates the activities of Se-

dependent antioxidant enzymes like glutathione peroxidase that could be due to elevated combination of selenocysteine which is substantial for their activities (Shaikh *et al.*, 1999).

In this respect, zinc exhibits antioxidative properties during exposure to cadmium through its ability to interact with various elements such as iron and copper reducing their content in tissues and diminishing the oxidative processes (Jemai, *et al.*, 2007). This trace element protactive reactive oxygen species production and is incorporated in cell membrane stabilization, superoxide dismutase (Cu/Zn SOD) and structure metallothionin (Mt) synthesis (Tandon, *et al.*, 2001).

Vitamin E addition enough to prevent the organism from toxic agent and free radical damage is a time consuming process. It is concluded that vit. E is an major compound of the kidney for prevention of this tissue against peroxidative damage (Champe and Harvey, 1987). Hanafy and Soltan (2004) reported the prevent effect of vit. E on some heavy meatalns such as Hg, cobalt, Pb, or nitrate induced rats. They found that the supplementation of vit. E and examined heavy metals can decrease the histological alteration and decrease creatinine and urea level in serum. Turguta *et al.* (2006) found that the supplementation of vit. E combined with aluminum (Al) lead to recovery of GSH and MDA concentration in mice supplemented with Al. That is, a chronic high dose of Al can lead to tissue oxidative injury, and vit. E can protecting the adverse effects of Al³⁺ ions. So, the observed normalization of glutathione peroxidase concentration, TAC and MAD following vit. E, Se or Zn supplement could be because these additives (antioxidants) played an action in diminishing the levels and accumulation of reactive oxygen species (ROS). While, vit. E supplemented with Se plus Zn suggested more effective as compared to individual alone.

CONCLUSION

Based on the results of the experiment, it is concluded that using polluted drainage water alone or mixed with Nile water in irrigation of agriculture land led to increasing the accumulation of cadmium in berseem causing possible hazardous effect in animal and human health. Contaminating of growing rabbit diets with cadmium revealed adverse effect on their productive performance, and mortality rate. Vitamin E, Se , Zn alone or combination have protective effect on Cd-induced oxidative stress. This study suggests that supplementation of vitamin E, Se , Zn alone or in combination may be a useful protective agent against the effect of the studied cadmium due to its antioxidant properties.

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التأثيرات الوقائية لأضافة فيتامين هـ - الزنك والسيلينيوم على اداء النمو وبعض القياسات البيوكيميائية للأرانب النامية المعرضة الى الكاديوم

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اجريت الدراسة الحالية في محافظة كفر الشيخ خلال موسم ٢٠١٦ لفحص تأثير استخدام ثلاثة انواع من المياه النوع الأول مياه النيل (ترعة ابو عمر) ، النوع الثاني عبارة عن خليط من مياه النيل ومياه الصرف الملوثة (ترعة البنا) أما النوع الثالث فكان مياه صرف ملوثة (كفر دخميس) وذلك في رى الأراضى الزراعية ثم دراسة تراكم الكاديوم في التربة والبرسيم الذى يزرع فيها. ثم تأثيره بعد ذلك على اداء النمو للأرانب التى تتغذى على علائق ملوثة بالكاديوم بنفس المستوى الموجود فى البرسيم. تم استخدام بعض المواد كمضادات لسوم الكاديوم مثل بعض مضادات الأكسدة مثل فيتامين هـ - زنك و سيلينيوم على ١٢٠ أرنب نامى من سلالة " ابرى ". تم تقسيم الأرانب الى ٦ مجاميع متساوية المجموعة الأولى تغذت على عليقة اساسية تحتوي على ٠.١ ملليجرام كاديوم/كجم علف على اساس المادة الجافة و المجموعة الثانية تغذت الأرانب على عليقة ملوثة بالكاديوم بمستوى نهائى على ٠.٩٦ ملليجرام كاديوم/كجم علف ، المجموعة الثالثة تغذت الأرانب على العليقة الملوثة مع اضافة فيتامين هـ (١٥٠ ملليجرام /كجم علف) ، المجموعة الرابعة تغذت الأرانب على عليقة ملوثة مع اضافة السيلينيوم (٠.٢ ملليجرام /كجم علف)، المجموعة الخامسة تغذت الأرانب على عليقة ملوثة مع اضافة الزنك (١٠٠ ملليجرام /كجم علف) اما المجموعة السادسة تغذت الأرانب على العليقة الملوثة مضاف اليها فيتامين هـ - سيلينيوم و زنك معا . اظهرت النتائج ان النوع الثالث من المياه سجل بصورة معنوية اعلى تركيز من الكاديوم بيلة النوع الثاني بينما كان النوع الأول اقل تركيزا. فى حين ان عينات البرسيم المنزرعة فى تربة تروى بمياه النوع الثالث كانت الأعلى معنويا فى محتوى الكاديوم بيلها البرسيم المنزرع فى تربة تروى بمياه النوع الثاني بينما البرسيم المنزرع فى تربة تروى بمياه النوع الأول اقل محتوى . اظهرت الأرانب المعاملة بالكاديوم نقص معنوى فى متوسط اداء النمو ومعامل هضم العناصر الغذائية بينما اضافة فيتامين هـ - زنك و سيلينيوم منفردة او معا حسنت معنويا كل قياسات النمو . لوحظت بعض التغيرات فى كل قياسات الدم فى مجموعة الأرانب التى تتناول عليقة ملوثة بالكاديوم فقط بينما كان لاضافة فيتامين هـ - زنك و سيلينيوم منفردة او معا الى العلائق الملوثة تأثير ايجابى ومعنوى على قياسات الدم . علاوة على ذلك نجد ان اضافة فيتامين هـ - زنك و سيلينيوم معا اظهرت كفاءة اكثر بالمقارنة بهذة الاضافات منفردة.