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AMELIORATION OF LEAD TOXICITY ON GROWING RABBITS

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ABSTRACT

The aim of the present study was to investigate the effect of the administration of vitamin C, clay, methionine and D-penicillamine against lead-induced toxicity. Rabbit groups fed diets contaminated with 200 mg lead/kg diet recorded the lowest final live body weight, weight gain and feed intake by 9.48, 13.03 and 10.57%, respectively when compared to the healthy control group, on the other hand, feed conversion ratio was impaired by 2.83%. Feed cost, return from body gain and final margin were decreased in rabbit fed diets contaminated with lead when compared with the control group. Lead ingestion resulted in an increase in plasma AST, urea-N and createnine, while the concentrations of hemoglobin, total protein, albumin, globulin and erythrocytes account were decreased.

Daily gain (0-8 weeks) were 53.63, 47.10, 61.32 and 63.16%, respectively in rabbit groups fed diets supplemented with clay, D-penicillamine, vitamin C and DL-methionine when compared with the control group. Rabbit groups fed diets supplemented with vitamin C and DL-methionine recorded the best feed conversion compared with the other experimental groups. Rabbit groups fed diets supplemented with vitamin C and DL-methionine recorded higher return from body gain and final margin than the other experimental groups.

Rabbits fed lead-exposed diets and supplemented with clay, D-penicillamine, vitamin C and DL-methionine recorded higher final body weight, daily gain, feed intake, return from body gain and final margin, and the best feed conversion compared with the control group. Administration of clay, D-penicillamine, vitamin C and DL-methionine in combination with lead significantly affected erythrocytes, hemoglobin, plasma total protein, urea-N, createnine and AST concentration.

Pre-slaughter weight affected each of significantly carcass, carcass cuts and kidney fat weights. On the other hand carcass weight and carcass components were insignificantly affected with lead toxicity, feed additives and their interactions.

Contaminated rabbit diets supplemented with clay, D-penicillamine, vitamin C and DL-methionine significantly ($P<0.001$) reduced lead residual in rabbits muscle. Lead residual in rabbit muscles fed lead contaminated diet was 1.917 mg/kg body weight, while the lead residual in rabbits fed diets contaminated with lead and supplemented with natural clay, D-penicillamine, vitamin C and DL-methionine were 1.293, 1.603, 1.407 and 1.433 mg/kg body weight, respectively.

Keywords: Rabbit, lead toxicity, rabbit, clay, methionine, vitamin C, D-penicillamine, growth performance, blood components, slaughter traits.

INTRODUCTION

Rabbit meat is routinely consumed in many European countries (Malta, Cyprus, Italy, Czech Republic, Spain, Belgium, Luxembourg, Portugal and France) and certain north African countries; Egypt and Algeria (FAO, 2012). In most of these nations, rabbit meat production plays an important role in the national economy.

Lead (Pb) is being a ubiquitous environmental contaminant due to its significant role in modern industry (Shalan *et al.*, 2005). Lead is a dangerous heavy metal which is widely spread in the environment. Lead content in the air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries (Tuormaa, 1995). Despite of attempts for reducing the exposure to this metal, there are still some reports of cases with severe lead toxicity (Roche *et al.,* 2005 and Coyle *et al.,* 2005). On the other hand, chronic lead poisoning is a problem which threats mankind's life and seems to be an unknown reason for some diseases during aging (Vig and Hu, 2000 and Coyle *et al*., 2005). Lead binds to sulfhydryl and amide groups, frequent components of enzymes, altering their configuration and diminishing their activities. It may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium.

Lead and its compounds play a significant role in modern industry; a wide variety of population was at risk of occupational exposure and lead is suspected to be a human carcinogen (Fracasso *et al*., 2002 and El Barbary *et al*., 2011). Lead has many undesired effects, including neurological (Royce *et al*., 1990), behavioral (Shafiq ur Rehman, 1991), respiratory (Hillam and Ozkan, 1986), growth retardation (Shukla *et al*., 1991), hematological (Falke and Xwennis, 1990), immunological (Sroczynski *et al*., 1987 and Ercal *et al.*, 2000), hepatic (Hao *et al*., 2002 and Patra and Swarup, 2004) and reproductive dysfunctions (Marchlewicz *et al*., 1993).

Lead is known to inhibit many enzyme activities and it may interfere with the synthesis of protein or RNA or both (Elayat and Bakheetf, 2010). The maximum acceptable toxicant limit for inorganic lead has been determined for several species under different conditions and results range from 0.04 mg/l to 0.198 mg/kg (WHO, 1995). The daily safe level for lead consumption in human food is 450 µg (WHO, 1972).

Vitamin C (ascorbic acid), as a chelating agent is reported in treatment of lead toxicity (Llobet et al., 1990). It reduces the possibility of lead interacting with critical biomolecules and factors inducing oxidative damage (Hsu and Guo, 2002). It acts as a free radical scavenger (Kleszczewska, 2001) and reduces the level of lipid peroxidation (Upasani et al., 2001). On the other hand, Simon and Hudes (1999) suggested that high serum levels of ascorbic acid are independently associated with a decreased prevalence of elevated blood lead levels; therefore its intake may have public health implications for control of lead toxicity.

D-Penicillamine (D-P) is a chelator drug which is used for treatment of lead toxicity for several years (Lyle. 1981). The efficacy of D-P in reducing blood lead level (BLL) has made it a good choice for treatment of chronic lead poisoning in adults (González-Ramírez *et al.,* 1990). D-P administration can increase the urinary excretion of lead because of complexes which it forms with this heavy metal. In many cases BLL fell down to acceptable range after D-P treatment. However, long period of administration and side effects of D-P have complicated its use in the treatment of lead poisoning (Shannon *et al., 1988).*

The present study aimed to investigate the effects of dietary lead contamination and its amelioration by using dietary clay, vitamin E and methionine supplementations on growth performance, carcass traits and blood components of growing male rabbits.

The aim of the present study was also to investigate the impact of the combined administration of vitamin C, clay, methionine and D-penicillamine with given individually on the same parameters in growing rabbits.

MATERIALS AND METHODS

The present study was conducted at a private farm at Met Abo-Khalid, Dakahlia Governorate. A total of 80 weaned New Zealand White (NZW) male rabbits of 28 days of age with nearly equal initial live body weight were used for 8 weeks. The rabbits were randomly allotted to 10 experimental groups (8 rabbits in each). The rabbits of the first five groups were fed the normal diets supplemented with feed additives without lead, while the other five groups were fed on the basal diet contaminated with 200 mg lead (lead oxide)/kg diet. Rabbit within each dietary group received lead level, the first group was fed the diet without supplementation the second was fed on the diet supplemented with clay (20 g/kg diet), the third group fed on the diet supplemented with 250 mg D-Penicillamine/kg diet, the fourth was fed on the diet supplemented with 50 mg vitamin C/kg diet and the fifth was fed on the diet supplemented with 2 g DL-methionine/kg diet.

All animals were fed a pelleted diet and watered *ad libitum*. Each kg of the basal diet consisted of 300 g alfalfa hay, 240 g corn, 130 g soybean meal, 280 g wheat bran, 30 g molasses, 14 g limestone, 3 g sodium chloride, 3 g vitamins and minerals premix. The diet contained 16.3% crude protein, 13.2% crude fibre (both were analyzed according to AOAC, 1990) and 11.2 Mj / kg digestible energy (calculated according to NRC, 1977).

All animals were kept under similar managerial and hygienic conditions, during the experimental period. The rabbits were raised in cages provided with feeders and automatic nipple drinkers. The building was naturally ventilated and provided with electric fans. Indoor ambient temperature and relative humidity values were recorded. Rabbits were individually weighed at the beginning and the end of the experimental period. Weighing was carried out before offering the morning meal at 8.00 h. Feed consumption was recorded during the experimental period and feed conversion was calculated.

At the end of the experimental period (8 weeks), four rabbits were chosen randomly from each group were slaughtered. After complete bleeding, pelt, viscera and tail were removed and the carcass and some carcass components (fore, intermediate and hind parts, liver and head), were weighed. Blood samples of rabbits were collected during slaughtering. Serum samples were obtained by centrifugation of blood at 3000 rpm for 20 minutes and kept at -20°C in deep freezer till further biochemical measurements.

Plasma total protein, albumin, creatinine, urea-N and tranaminase enzymes were determined by using commercial kits. Also, three milliliters of blood was collected from each rabbit in a sterile glass test-tube. The samples were then stored at +4 °C and processed in the laboratory on the same day. The total count of erythrocytes and the haemoglobin concentration were performed using a haematological meter.

The lead contents in the tissues were determined by using the atomic absorption spectrophotometric technique according to Nation and Robinson (1971). About 0.3 – 0.5 g of each sample was digested by using 5 ml of acid mixture (nitric acid: perchloric acid : sulfuric acid 3 : 2 : 1, respectively) in long necked Kjeldahl flask. The cooled acids mixture was added to the sample and heated slowly to encourage smooth digestion without charring. When the contents of the flask become colorless, the flasks were cooled and the contents were transferred quantitavely in a 25 ml volumetric flask with deionizer water.

Economic evaluation was calculated as the following equation (Ayyat, 1991), Margin = Return from body gain weight - Feed cost. Other overhead costs were assumed constant. Price of one kg of diet was 2.225 LE and price of selling of one kg live body weight of rabbits was 18.0 LE.

The data of body weight, daily body gain and blood components of rabbits were statistically analyzed by completely randomize experiment (SAS, 2002) according to the following Model 1: $Y_{ij} = \mu + T_i e_{ij}$, where Yij = an observation, μ = the overall mean, T_i = the fixed effect of i^{th} treatment (i= 1, ..5), and e_{ij} = random error. The data of slaughter traits were statistically analyzed by analysis of covariance according to the following Model 2: $Y_{ij} = \mu + T_i + b(X-x) + e_{ij}$, where, Yijk, μ, T_i, and e_{ij} were as defined in the Model 1, b = partial linear regression coefficients of Y_{ij} on slaughter weight, X = value of slaughter weight and x = overall average of slaughter weight. Significant differences were determined by Duncan's Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance:

Live body weight and daily gain of growing New Zealand White rabbits as affected by lead toxicity, some feed additives and their interactions are illustrated in Table 1. Live body weight and daily body gain of male rabbits decreased significantly (P<0.001) with lead pollution. Rabbit groups fed diets contaminated with 200 mg lead/kg diet recorded the lowest final live body weight and weight gain (0-8 weeks) with 9.48 and 13.03%, respectively when compared to the healthy control (rabbit groups fed diets without lead pollution). Similar results were obtained by Shehata (2011), who found that the live body weight and daily body weight gain were decreased (P<0.05) significantly by addition of lead acetate as compared to the healthy control. The reduction in live body weight as affected with the lead pollution may be related either to change in behavior of eating habbit through the inhibition of the hypothalamic appetite center (Cragg and Ress, 1984) or to alteration in digestive enzyme secretion (Deborah *et al*., 1980). This may cause increased permeability of the cells and damage or even death of those cells. Lead can displace calcium in bone, deposit there and form softer bone, lead binds with the sulfhydryl bonds and inactivates the cysteine-containing enzymes, which allows more internal toxicity from free radicals, chemicals, and other heavy metals. Lead is also an immunosuppressant; it lowers host resistance to bacteria and viruses, and thus allows increase infection susceptibility (Hass, 1992).

Table 1. Live body weight and gain of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

Items	Live body weight			Daily gain (g/day)		
	0 W	4 W	8 W	0-4 W	4-8 W	0-8 W
Effect of lead contamination						
0 mg	529.1±1.81	1194.5±17.97a	1888.1±28.14a	23.763±0.621a	24.772±0.598a	24.268±0.493a
200 mg	527.2±1.76	1080.3±17.09b	1709.2±41.81b	19.750±0.631b	22.462±1.072b	21.107±0.755b
P value	0.5063	0.0001	0.0001	0.0001	0.0002	0.0001
Effect of feed additives						
Control	527.3±3.81	964.0±21.61^{c}	1402.7±53.69^{d}	15.600±0.801^{c}	15.67±1.448^{c}	15.63±0.984^{d}
Clay	527.8±3.06	1171.6±25.81^{ab}	1872.5±22.18^{b}	22.991±0.882^{b}	25.033±0.591^{ab}	24.012±0.378^{b}
Penicillamine	527.5±2.28	1154.7±15.56^{b}	1815.0±16.68^{c}	22.399±0.567^{ab}	23.583±0.693^{b}	22.991±0.298^{c}
Vitamin C	529.0±2.30	1188.0±19.61^{ab}	1941.0±26.48^{ab}	23.536±0.708^{ab}	26.893±0.861^{a}	25.214±0.473^{a}
Methionine	529.4±2.70	1208.1±27.12^{a}	1957.5±30.82^{a}	24.241±0.967^{a}	26.763±0.876^{a}	25.502±0.546^{a}
P value	0.9848	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction effect of lead contamination and feed additives						
0 mg Lead						
Control	522.5±5.59	1021.9±22.40^{e}	1589.4±9.47^{d}	17.835±0.764^{d}	20.268±0.949^{c}	19.052±0.188^{d}
Clay	531.9±5.26	1260.0±21.46^{a}	1936.9±20.51^{b}	26.005±0.710^{a}	24.174±0.953^{b}	25.089±0.338^{b}
Penicillamine	530.6±2.58	1170.0±25.91^{bc}	1831.3±21.40^{c}	22.835±0.906^{b}	23.616±0.708^{b}	23.226±0.363^{c}
Vitamin C	528.8±3.63	1231.3±25.53^{ab}	2014.4±17.61^{a}	25.089±0.911^{a}	27.969±1.016^{a}	26.529±0.300^{a}
Methionine	531.9±1.88	1289.4±16.49^{a}	2068.8±16.84^{a}	27.054±0.595^{a}	27.835±0.827^{a}	27.444±0.302^{a}
200 mg Lead						
Control	532.9±4.61	897.9±17.32^{f}	1189.3±8.12^{e}	13.036±0.616^{e}	10.408±0.798^{d}	11.722±0.120^{e}
Clay	523.8±2.95	1083.1±12.64^{de}	1808.1±22.44^{c}	19.978±0.484^{a}	25.893±0.616^{ab}	22.935±0.407^{c}
Penicillamine	524.4±3.59	1139.4±17.28^{cd}	1798.8±25.67^{c}	21.964±0.709^{bc}	23.549±1.247^{b}	22.757±0.483^{c}
Vitamin C	529.3±2.97	1138.6±18.70^{cd}	1857.1±29.90^{c}	21.760±0.649^{bc}	25.663±1.362^{ab}	23.712±0.542^{c}
Methionine	526.9±5.08	1126.9±31.54^{cd}	1846.3±15.83^{c}	21.429±1.180^{bc}	25.692±1.510^{ab}	23.560±0.330^{c}
P value	0.1602	0.0087	0.0001	0.0095	0.0001	0.0001

Means in the same column within each classification with different letters differ significantly (P<0.05).

Live body weight and daily gain weight were affected significantly (P<0.001) with the feed additives (Table 1). Rabbits fed diets supplemented with tested feed additives recorded higher live body weight and daily gain than the rabbit fed diets without supplementation. Final body gain increased with 33.45, 29.39, 38.38 and 39.55%, respectively in rabbit groups fed diets supplemented with clay, D-penicillamine, vitamin C and DL-methionine; when compared with the control group. The corresponding figures for daily gain (0-8 weeks) were 53.63, 47.10, 61.32 and 63.16%, respectively. Rabbit groups fed diets supplemented with vitamin C and DL-methionine recorded higher final body weight and growth rate than the other experimental groups (Table 1).

The interaction between lead pollution and feed additives affected significantly (P<0.001 or 0.01) live body weight and daily gain (Table 1). Rabbits fed lead-exposed diets and supplemented with clay, D-penicillamine, vitamin C and DL-methionine recorded higher final body weight and daily gain than the control group (fed healthy diet without feed additives). Combined treatment of lead-exposed rabbits with clay, D-penicillamine, vitamin C and DL-methionine showed marked improvement of the growth rate. These experimental results strongly indicate the protective effect of vitamin C and DL-methionine against toxic effects of lead on final body weight and daily gain. The preventive activity of vitamin C may relate to its antioxidant efficacy that inhibits lipid peroxidation enhanced by lead (Upasani *et al*., 2001). Shehata (2011) reported that methionine addition significantly (P<0.05) improved daily body weight gain compared with those fed lead diet alone.

The beneficial effect of methionine may be due to increase of feed intake and nutritive values as a result of its content, reducing the binds of lead by sulfur groups on protein of enzymes, hormones and cell receptor by increase lead excretion in feces and urine (Paredes *et al*.,1985 and Flora and Seth, 1999). Also, the beneficial effect of D-Penicillamine administration can increase the urinary excretion of lead because of complexes which it forms with this heavy metal (González-Ramírez *et al*., 1990).

Feed efficiency:

Daily feed intake decreased by 10.57% in rabbit group fed diets contaminated with lead when compared with the control group, on the other hand feed conversion ratio impaired by 2.83% (Table 2). Similar results were obtained by Fathi *et al*. (1999) and Shehata (2011).

Table 2. Feed intake, feed conversion and profit analysis of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

	Feed intake (g/day)	Feed conversion (g food/g gain)	Feed cost (LE/rabbit)	Return from gain (LE/rabbit)	Final margin (LE/rabbit)	Lead residual (mg/kg) in muscle*
Effect of lead contamination						
0 mg	104.66	4.313	13.041	24.462	11.422	ND
200 mg	93.60	4.435	11.663	21.276	9.613	1.531±0.0619
P value	-----	-----	-----	-----	-----	0.0001
Effect of feed additives						
Control	93.00	5.950	11.588	15.755	4.167	0.958±0.429[a]
Clay	47.85	4.102	12.273	24.204	11.931	0.647±0.290[c]
Penicillamine	101.00	4.393	12.585	23.175	10.590	0.802±0.350[b]
Vitamin C	101.50	4.026	12.647	25.416	12.769	0.703±0.3148[c]
Methionine	101.65	3.986	12.666	25.706	13.040	0.717±0.323[c]
P value	-----	-----	-----	-----	-----	0.0001
Interaction effect of lead contamination and feed additives						
0 mg Lead						
Control	106.50	5.590	13.270	19.204	5.935	ND
Clay	101.30	4.038	12.622	25.290	12.668	ND
Penicillamine	105.10	4.525	13.095	23.412	10.316	ND
Vitamin C	104.90	3.954	13.071	26.741	13.671	ND
Methionine	105.50	3.844	13.145	27.664	14.518	ND
200 mg Lead						
Control	79.50	6.782	9.906	11.816	1.910	1.917±0.0449[a]
Clay	95.70	4.173	11.924	23.118	11.194	1.293±0.030[d]
Penicillamine	96.90	4.258	12.074	22.939	10.865	1.603±0.073[b]
Vitamin C	98.10	4.137	12.223	23.902	11.678	1.407±0.032[cd]
Methionine	97.80	4.151	12.186	23.748	11.563	1.433±0.0841[c]
P value	-----	-----	-----	-----	-----	0.0001

*** Means in the same column within each classification with different letters differ significantly (P<0.05).**
ND: not detected (0.000 mg)

Fathi *et al*., (1999) reported that 200-400 ppm lead chloride in feed of broiler chicks led to poor feed conversion. Also, Shehata (2011) reported that the average daily feed intake and feed conversion were decreased (P<0.05) significantly by addition of lead acetate as compared to the healthy control.

Rabbits fed diets supplemented with tested feed additives increased daily feed intake and improved feed conversion when compared with the rabbit fed diets without supplementation (Table 2). Rabbit groups fed diets supplemented with vitamin C and DL-methionine recorded the best feed conversion than the other experimental groups.

The present experimental results indicate the protective effect of clay, D-penicillamine, vitamin C and DL-methionine against toxic effects of lead on daily feed intake and feed conversion (Table 2). Rabbits fed diets contaminated with lead and supplemented with clay, D-penicillamine, vitamin C or DL-methionine recorded higher feed intake and the best feed conversion when compare with those fed diets contaminated with lead or those fed healthy diets (without lead contamination). The beneficial effect of D-penicillamine, vitamin C and DL-methionine may be due to increase of feed intake and improved feed conversion. The ability of vitamin C to reduce lead toxicity may relate to its antioxidant actions via free radical scavenging mechanism. Supplementation of diets with vitamin C may be recommended to improve the body burden of lead (Raafat *et al*., 2009).

Economic efficiency:

Feed cost, return from body gain and final margin were decreased in rabbit fed diets contaminated with lead when compared with the control group (Table 2). The decreasing in final margin was attributed to the decrease in body gain rate in rabbits fed diets contaminated with lead.

Feed cost, return from body gain and final margin increased in rabbit fed diets supplemented with feed additives when compared with the rabbit group fed diets without feed additives (Table 2). Rabbit groups fed diets supplemented with vitamin C and DL-methionine recorded higher return from body gain and final margin than the other experimental groups.

Rabbits fed diets contaminated with lead and supplemented with clay, D-Penicillamine, vitamin C and DL-methionine recorded higher return from body gain and final margin when compared with those fed diets contaminated with lead or those fed healthy diets (Table 2). Rabbits fed diets contaminated with lead and supplemented with vitamin C and DL-methionine recorded higher return from body gain and final margin.

Blood components:

This study clearly showed that lead acetate ingestion with a concentration of 200 mg/kg diet induced a significant (P<0.001 or 0.01) elevation of erythrocytes and hemoglobin concentration and plasma total protein, albumin, globulin, AST and ALT concentrations (Table 3 and 4). Lead ingestion resulted in an increase in plasma AST, urea-N and createnine, while the concentrations of each hemoglobin, total protein, albumin, globulin and erythrocytes count. Increasing the level of AST in the serum of rabbits fed diets contaminated with lead may reflect the impaired to the liver functions due to its role of lead detoxification and decreasing the protein synthesis in the liver. The reduction in the concentrations of blood total protein and albumin indicates the impaired protein synthesis in the liver (Khan *et al*., 1993). Hassanin (1994) has reported that serum ALT was elevated significantly more than AST on lead exposure. The depression in the concentrations of total protein and albumin in the hen blood may be the reason for the reduction in the growth rate. Also, Abou-Zeid *et al*. (2000) indicated a sharp decrease in total protein, albumin and globulin in ducks fed 400 mg lead

Administration of clay, D-penicillamine, vitamin C and DL-methionine in rabbit diets significantly (P<0.001 or 0.01) improved erythrocytes and hemoglobin, plasma total protein, albumin, globulin, and ALT concentrations (Table 3 and 4). On the other hand, the concentrations of urea-N and AST were significantly (P<0.05) decreased in rabbit fed diets supplemented with the experimental feed additives.

Table 3. Plasma total protein and its fractions, red blood cells count and hemoglobin concentration of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

Items	Total Protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	Erythrocytes (10^{12}/l)	Hemoglobin (g/l)
Effect of lead contamination					
0 mg	6.717±0.093a	3.762±0.067a	2.955±0.046a	5.76±0.034a	129.3±0.86a
200 mg	6.218±0.144b	3.476±0.098b	2.742±0.067b	4.71±0.071b	118.1±1.55b
P value	0.0001	0.0009	0.0021	0.0001	0.0001
Effect of feed additives					
Control	5.595±0.207^{b}	3.063±0.125^{b}	2.533±0.125^{b}	4.94±0.297^{c}	115.1±3.63^{c}
Clay	6.515±0.127^{a}	3.660±0.119^{a}	2.855±0.014^{a}	5.18±0.184^{b}	122.0±1.99^{b}
Penicillamine	6.698±0.111^{a}	3.785±0.094^{a}	2.913±0.074^{a}	5.33±0.178^{ab}	126.3±1.76^{a}
Vitamin C	6.730±0.098^{a}	3.826±0.074^{a}	2.904±0.068^{a}	5.41±0.162^{a}	127.3±1.99^{a}
Methionine	6.798±0.141^{a}	3.759±0.091^{a}	3.039±0.082^{a}	5.31±0.196^{ab}	127.9±1.78^{a}
P value	0.0001	0.0001	0.0004	0.0001	0.0001
Interaction effect of lead contamination and feed additives					
0 mg lead					
Control	6.108±0.041^{d}	3.350±0.102	2.758±0.067	5.73±0.072^{a}	124.5±1.32^{bc}
Clay	6.703±0.196^{abc}	3.820±0.188	2.883±0.017	5.65±0.090^{a}	127.0±1.08^{b}
Penicillamine	6.755±0.172^{abc}	3.790±0.125	2.965±0.083	5.78±0.085^{a}	130.5±1.32^{a}
Vitamin C	6.945±0.068^{ab}	3.940±0.045	3.005±0.083	5.82±0.074^{a}	132.3±1.25^{a}
Methionine	7.073±0.117^{a}	3.908±0.057	3.165±0.137	5.81±0.062^{a}	132.3±1.25^{a}
200 mg lead					
Control	5.083±0.145^{e}	2.775±0.085	2.308±0.188	4.16±0.021^{d}	105.8±1.11^{e}
Clay	6.328±0.116^{cd}	3.500±0.117	2.828±0.013	4.71±0.057^{c}	117.0±0.82^{d}
Penicillamine	6.640±0.162^{abc}	3.780±0.161	2.860±0.131	4.88±0.077^{bc}	122.0±1.08^{c}
Vitamin C	6.415±0.097^{bcd}	3.713±0.121	2.803±0.090	5.00±0.060^{b}	122.3±0.63^{c}
Methionine	6.523±0.168^{bcd}	3.610±0.144	2.913±0.043	4.81±0.093^{bc}	123.5±0.65^{c}
P value	0.0357	0.2645	0.3384	0.0001	0.0002

Means in the same column within each classification with different letters differ significantly (P<0.05).

Administration of clay, D-penicillamine, vitamin C and DL-methionine in combination with lead significantly (P<0.001, 0.01 or 0.05) effected erythrocytes, hemoglobin, plasma total protein, urea-N, createnine and AST

(Table 3 and 4). Vitamin C proved its antioxidant effect on recuperating the normal status of enzymes in serum (El-Tohamy and El-Nattat, 2010).

Table 4. Plasma urea-N, createnine, aspartate amino transferase (AST) and alanine amino transferase (ALT) of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

Items	Urea-N (mg/100ml)	Createnine (mg/100ml)	AST (U/L)	ALT (U/L)
Effect of lead contamination				
0 mg	14.865±0.232	1.085±0.012	29.965±0.322b	15.225±0.337a
200 mg	15.160±0.406	1.111±0.019	36.620±0.304a	13.965±0.247b
P value	0.3709	0.1343	0.0001	0.0012
Effect of feed additives				
Control	16.213±0.768[a]	1.126±0.039	33.988±1.797[a]	13.413±0.373[b]
Clay	14.325±0.206[b]	1.080±0.025	33.563±1.026[a]	14.113±0.342[ab]
Penicillamine	14.763±0.517[b]	1.078±0.019	32.213±1.468[b]	15.325±0.468[a]
Vitamin C	14.863±0.434[b]	1.123±0.017	33.513±1.128[a]	15.288±0.485[a]
Methionine	14.900±0.348[b]	1.084±0.017	33.188±1.129[ab]	14.838±0.608[a]
P value	0.0127	0.1893	0.0374	0.0067
Interaction effect of lead contamination and feed additives				
0 mg lead				
Control	14.275±0.345[b]	1.038±0.019[c]	29.325±0.409[cd]	13.775±0.638
Clay	14.350±0.189[b]	1.048±0.023[c]	30.925±0.063[c]	15.000±0.041
Penicillamine	14.925±0.811[b]	1.070±0.009[b]	28.500±0.851[d]	14.275±0.994
Vitamin C	15.700±0.422[b]	1.153±0.010[a]	30.625±0.528[c]	16.050±0.659
Methionine	15.075±0.511[b]	1.118±0.017[b]	30.450±0.849[c]	16.025±0.776
200 mg lead				
Control	18.150±0.366[a]	1.215±0.040[a]	38.650±0.640[a]	13.050±0.393
Clay	14.300±0.402[b]	1.113±0.040[bc]	36.200±0.520[b]	13.225±0.138
Penicillamine	14.600±0.756[b]	1.085±0.040[bc]	35.925±0.371[b]	15.375±0.189
Vitamin C	14.025±0.482[b]	1.093±0.027[bc]	36.400±0.319[b]	14.525±0.525
Methionine	14.725±0.534[b]	1.050±0.017[c]	35.925±0.484[b]	13.650±0.428
P value	0.0001	0.0004	0.0039	0.2278

Means in the same column within each classification with different letters differ significantly (P<0.05).

Slaughter traits:

Pre-slaughter weight affected significantly (P<0.001, 0.01 or 0.05) carcass, carcass cuts and kidney fat weights. On the other hand, carcass weight and carcass components were insignificantly affected with lead toxicity, feed additives and their interactions (Table 5).

Lead residual in muscles:

Tissue lead residues were affected significantly (P<0.001) with experimental treatments (Table 2). Rabbit group fed diet contaminated with lead recorded higher (P<0.001) lead residual in muscle when compared with the rabbits fed diets without lead contamination. Supplementing contaminated rabbit diets with clay, D-penicillamine, vitamin C or DL-methionine significantly (P<0.001) reduced lead residual in rabbits muscle. Lead residual in rabbit muscles fed contaminated diet with lead was 1.917 mg/kg body weight, while the lead residual in rabbits fed diets contaminated with lead and supplemented with natural clay, D-penicillamine, vitamin C and DL-methionine the lead residual were 1.293, 1.603, 1.407 and 1.433 mg/kg body weight, respectively.

Table 5. Actual pre-slaughter live weight and adjusted carcass, liver and kidney fat weight of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

Items	Pre-slaughter weight (g)	Carcass weight (g)	Liver weight (g)	Kidney fat weight (g)
Effect of lead contamination				
0 mg	2032.8±18.29a	1109.4±17.24	68.31±6.067	19.409±1.257
200 mg	1720.0±22.82b	1087.65±17.24	59.94±6.067	17.991±1.27
P value	0.0001	0.5143	0.4759	0.5595
Effect of feed additives				
Control	1805.0±98.56[b]	1085.2±13.37	66.84±4.708	18.978±0.975
Clay	1916.3±77.79[a]	1104.0±11.98	66.83±4.219	19.314±0.874
Penicillamine	1866.9±42.49[a]	1107.9±11.34	65.11±3.991	18.288±0.827
Vitamin C	1878.8±49.21[a]	1096.7±11.30	61.91±3.978	17.959±0.824
Methionine	1915.0±45.79[a]	1098.7±11.94	59.93±4.204	18.960±0.871
P value	0.0037	0.7538	0.7001	0.7702
Interaction effect of lead contamination and feed additives				
0 mg lead				
Control	2062.5±7.77[b]	1085.7±24.57	69.70±8.650	18.548±1.792
Clay	2118.8±12.97[a]	1115.5±29.09	71.01±10.241	21.830±2.121
Penicillamine	1968.8±35.61[c]	1114.9±18.47	72.59±6.502	18.66±1.347
Vitamin C	1993.8±45.16[bc]	1109.5±19.84	65.45±6.986	18.73±1.447
Methionine	2020.0±48.69[bc]	1121.5±21.51	62.83±7.573	19.28±1.569
200 mg lead				
Control	1547.5±32.76[f]	1084.8±36.65	63.99±12.903	19.408±2.673
Clay	1713.8±27.03[e]	1092.6±22.83	62.64±8.038	16.798±1.665
Penicillamine	1765.0±15.41[de]	1100.9±19.50	57.64±6.863	17.916±1.422
Vitamin C	1763.8±21.15[de]	1084.0±19.57	58.37±6.888	17.188±1.427
Methionine	1810.0±7.91[d]	1076.0±17.31	57.04±6.093	18.642±1.262
P value	0.0001	0.8152	0.9318	0.1599
LW-P value	-------	0.0001	0.6847	0.0257

LW-P value = Regression on slaughter weight.
Means in the same column within each classification with different letters differ significantly (P<0.05).

Mariam *et al*. (2004) reported mean levels of 2.18, 4.25 and 3.15 mg kg^{-1} for lead in beef, mutton and poultry, respectively. The levels found in this study were much higher than these values and were under the present study. The contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006). These pollutants often have direct physiological toxic effects because they are stored or incorporated in tissues, sometimes permanently (Bokori *et al*., 1996 and Mariam *et al*., 2004).

Table 6. Adjusted carcass components of New Zealand White male rabbits as affected by lead toxicity, some feed additives and their interactions

Items	Head Weight (g)	Fore part Weight (g)	Intermediate Weight (g)	Hind part Weight (g)	Prime cuts Weight (g)
Effect of lead contamination					
0 mg	113.4±3..51	245.9±6.21	285.2±10..31	364.1±9.95	649.3±15.75
200 mg	119.3±3.51	240.4±6.21	278.7±10.31	357.1±9.95	636.1±15.75
P value	0.3903	0.6422	0.7533	0.7162	0.6636
Effect of feed additives					
Control	116.6±2.72	236.0±4.82	276.9±8.00	353.9±7.72	630.8±12.22
Clay	115.9±2.44	244.0±4.32	275.4±7.17	369.4±6.92	644.7±10.95
Penicillamine	116.5±2.31	244.5±4.09	283.7±6.78	367.6±6.54	651.3±10.36
Vitamin C	117.3±2.30	247.1±4.07	282.0±6.76	357.0±6.52	639.1±10.33
Methionine	115.4±2.43	244.0±4.30	292.4±7.14	355.2±6.89	647.6±10.91
P value	0.9859	0.5229	0.4622	0.3488	0.7482
Interaction effect of lead contamination and feed additives					
0 mg lead					
Control	105.1±5.01	232.9±8.86	273.5±14.69	372.1±14.18	645.6±22.45
Clay	111.4±5.93	251.3±10.48	280.3±17.40	366.8±16.79	647.1±26.58
Penicillamine	119.5±3.76	241.2±6.66	288.3±11.04	362.4±10.66	650.7±16.88
Vitamin C	117.3±4.04	255.0±7.15	282.1±11.87	357.3±11.45	639.5±18.14
Methionine	113.8±4.38	249.3±7.75	301.8±12.86	362.0±12.42	663.7±19.66
200 mg lead					
Control	128.1±7.47	239.1±13.21	280.3±21.92	335.8±21.15	616.1±33.49
Clay	120.4±4.65	236.8±8.23	270.4±13.65	371.9±13.18	642.3±20.86
Penicillamine	113.4±3.97	247.9±7.03	279.2±11.66	372.7±11.25	651.9±17.82
Vitamin C	117.3±3.99	239.3±7.05	281.9±11.70	356.7±11.29	638.6±17.88
Methionine	117.0±3.53	238.7±6.24	282.9±10.35	348.5±9.99	631.5±15.82
P value	0.0517	0.1569	0.8024	0.2155	0.6816
LW-P value	0.0002	0.0003	0.0029	0.0014	0.0002

Means in the same column within each classification with different letters differ significantly (P<0.05).

In conclusion, the combined administration of lead-exposed rabbits with clay (20 g/kg diet), D-penicillamine (250 mg/kg diet), vitamin C (50 mg/kg diet) and DL-methionine (2 g/kg diet) confer significant protection against lead toxicity, but vitamin C and DL-methionine showed marked improvement of the growth rate and feed conversion.

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تخفيف سمية الرصاص على الأرانب النامية

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كان الهدف من هذه الدراسة بحث تأثير سميةالرصاص وكلا من فيتامين ج ومعادن الطين (الطفلة)، والميثيونين و د-بنيسيلامين على الأرانب النامية . تم تقسيم الأرانب إلى مجموعتين الأولى تغذت على علف غير ملوث والمجموعة الثانية تغذت على علف ملوث بـ ٢٠٠ ملج رصاص/كجم. الأرانب التى تغذت على علف ملوث بالرصاص سجلت أدنى وزن جسم نهائي وكذلك أقل معدل نمو وأقل كمية غذاء متناول بحوالى ٩,٤٨، ١٣,٠٣ و ١٠,٥٧%، على التوالي بالمقارنة مع مجموعة المقارنة الصحية التى تغذت على علف غير ملوث. من ناحية أخرى معدل التحويل الغذائى تدهور بنسبة ٢,٨٣%. تكلفة التغذية والعائد من النمو وهامش الربح النهائي انخفض في الأرانب التى تغذيت على علف ملوث بالرصاص عند مقارنة مع مجموعة المقارنة. التغذية على علف ملوث أدى إلى زيادة في AST واليوريا وكرياتينين البلازما ، بينما تركيزات كل من الهيموجلوبين والبروتين الكلي والألبومين والجلوبيولين والكريات الحمراء انخفضت.

معدل النمو اليومي (٠-٨ أسابيع) ارتفعت بمعدل ٥٣,٦٣ ٤٧,١٠ و ٦١,٣٢ و ٦٣,١٦%، على التوالي في مجموعات الأرنب التى تغذت على علف مزود بفيتامين ج بكلا من الطين و د- بنيسيلامين وفيتامين ج الميثيونين؛ عند مقارنة مع مجموعة المقارنة. مجموعات الأرنب التى تغذيت على علف مزود بفيتامين ج وميثيونين سجلت أفضل معدل تحويل غذائى عند المقارنة مع المجموعات التجريبية الأخرى. مجموعات الأرانب تغذت على علف مزود بكلٍ من فيتامين ج وميثيونين سجلت أعلى عائد من نمو الجسم وهامش الربح النهائي عن المجموعات التجريبية الأخرى.

الأرانب التى غذيت على علف ملوث بالرصاص مع إضافة كلا من الطين، د- بنيسيلامين وفيتامين ج والميثيونين سجلت أعلى وزن جسم نهائي وكذلك أعلى معدل نمو والعائد من النمو وهامش الربح النهائى.إضافة كلٍ من الطين وفيتامين ج و د- بنيسيلامين والميثيونين أدى إلى زيادة كبيرة فى عدد كريات الدم الحمراء والهيموجلوبين والبروتين الكلي في بلازما الدم. وزن قبل الذبح أثر معنوياً كلا من وزن الذبيحه، وكذلك قطعيات الذبيحة ودهون الكلى. من ناحية أخرى وزن الذبيحة، ومكونات الذبيحة لا تتأثر بالرصاص أو الإضافات الغذائية أو التفاعل بينهم.

إضافة الطين وفيتامين ج و د- بنيسيلامين والميثيونين إلى علف الأرانب الملوث بالرصاص قلل بقايا الرصاص في عضلات الأرانب.

قام بتحكيم البحث

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