J. Agric. Sci. Mansoura Univ., 27 (12): 8251 - 8265, 2002

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON LAYERS EXPOSED TO LEAD AND THE ROLE OF NATURAL CLAY IN PREVENTING ADVERSE EFFECTS AND REDUCING LEAD RETENTION

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ABSTRACT

This study was conducted to investigate the effect of lead acetate-polluted diet ingestion on some biochemical, immunological and physiological parameters of laying hens, and try to employ some useful properties of clay for reducing the adverse effect of lead and its accumulation in eggs and tissues. For this purpose 180 Gimmizah laying hens at 38 weeks were randomly distributed equally into nine treatments:- control, control+250ppm pb, control+250ppm pb+2.5% clay, control+250ppm pb+5% clay, control+500ppm pb, control+500ppm pb+2.5% clay, control+500ppm pb+5% clay, control+2.5% clay and control+5% clay. Blood total protein, albumin, globulin, total calcium, phosphorus and the activity of some enzymes (GOT, GPT and ALP) as well as egg, liver and muscle lead residues were determined for two times. The first time after the positive experimental period, the second after the negative experimental period. Also hemoglobin and antibody titer were determined for one time only (After the first). Results obtained for the first period indicated that, lead-polluted diets significantly decreased plasma total protein, total calcium, hemoglobin, GPT, GOT and ALP activity and antibody titer values. However, the levels of albumin, globulin and phosphorus were not significantly affected by feeding lead polluted diets. Lead residuals were accumulated in a dose-dependent manner. The highest lead deposition occurred in liver, while the lowest was in muscles. Adding clay to lead-polluted diets significantly reduced the adverse effect of lead on the previous parameters and reduced lead retention in egg, liver and muscles. After the negative experimental period all the previous parameters were go back around their normal ranges. The concentrations of lead residue in egg, liver and muscles were reduced to that of the control group.

It could be recommended that, adding the natural clay at level of 5 % to layer diet contaminated with lead can prevent the adverse effect of lead .

Keywords : layers – lead – clay – blood – constituent .

INTRODUCTION

To date, one of the most important environmental issues is feed contamination. Lead is considered one of the major environmental toxicant pollutants. It is well known that it causes hemolysis via destruction the cell membrane of red blood cells and it had an adverse effect on hematological and biochemical parameters (Khan *et al.,* 1993). The effect of lead on chicken, dove and wild birds is well documented (Kendall and Scanlon, 1981 and DiGuilio and Scanlon, 1984). Furthermore, the ingestion of lead by laying birds resulted in an increase of lead concentration in their eggs and muscular tissues (Finely *et al*., 1976 and Mazliah *et al.,* 1989).

Natural clay (Crystalline aluminosilicates) is characterized by its ability to exchange cations without major change in structure. It also can, adsorb toxic products of digestion. As a result of decreasing the accumulation of toxic substances in tissues, the incidence of internal disorders was reduced (Mumpton and Fishman, 1977). The information about the role of natural clay on blood constituents, egg production and lead accumulation in muscles when it added to lead-polluted rations is paucity. The objectives of this study were to investigate the effect of adding different levels of lead and/or natural clay to laying hen diets on some blood parameters, antibody immune response, and to find out the possibility of employing natural clay in decreasing the lead accumulation in egg and muscles of laying hens.

MATERIALS AND METHODS

The present study was carried out at Gimmizah Poultry Breeding Station, Animal Production Research Institute.

A total number of 180 Gimmizah laying hens at 38 weeks old were randomly distributed into nine groups of 20 hens in each group. All birds were individually housed in wired-floor batteries and maintained under the same managerial hygienic and environmental conditions for an experimental period of 8 weeks.

Birds had free access to feed and water and were maintained under 16L 8D lighting regime. The control group was fed the basal diet (a commercial laying ration containing 16.05% C.P and 2726.76 kcal ME/kg, Table 1a). Composition of the natural clay is shown in Table 1b. The experimental groups were fed the basal diet supplemented with different levels of clay and lead (in a lead acetate form). The experimental design is presented in Table 2.

Table (1a): Compostion of the basal diet.

Ingredient	**%**
Yellow corn	65.40
Soya bean meal	22.00
Wheat bran	3.00
Di-Cal-ph	1.39
Lime stone	7.44
Salt (NaCl)	0.30
Vit + Minerals	0.30
DL-Methionine	0.17
Total	100
Calculated analysis	
Crude protein (%)	16.05
ME (kcal)	2726.76
Crude fibers (%)	3.37
Ca (%)	3.20
Total ph (%)	0.62
Lysine (%)	0.82
Methionine (%)	0.45

Each 2.5 kg of vit. & min. mixture contain: vit A 12000.000 IU, vit D_3 2000.000 IU, vit E 10.000 mg, vit k_3 2000 mg, vit B_1 1000 mg, vit B_2 4000 mg,vit B_6 1500 mg, vit b_{12} 10 mg, Niacin 50.000 mg, Pantothenic acid 10.000 mg,Choline chloride 500.000, Copper 10.000 mg, Iodine 1.000 mg, Iron 30.00 mg, Manganese 55.000 mg, Zinc 55.000 mg and Selenium 100 mg.

Table (1b): Composition of the natural clay.

Item	%
SiO_2	50-55
$Al2O_3$	18-22
Fe_2O_3	3-5
Na_2O	2.0-3.2
CaO	0.1-0.3
MgO	0.5-1.6
K_2O	1.2-2.2
L.O.I	8-14
Moisture content (110 °C)	10.0 max
Surface area (m^2/gm) of raw are.	27
Mineralogical Composition	**%**
Montmorillonite	72->75
Kaolinite	<8
Non-clay	<5

Table (2): The experimental design (experimental groups and diets).

Experimental groups	Experimental diet	
	Supplemented level of lead (ppm)	Supplement level of clay (%)
A	250	-
B	250	2.5
C	250	5
D	500	-
E	500	2.5
F	500	5
G	-	2.5
H	-	5

The 8 weeks experimental period was divided into two equal period. The positive experimental period (P.Exp.P), in which birds were fed the experimental rations and the negative experimental period (N.Exp.P) in which all experimental group were fed the basal di*et al*one.

Blood plasma biochemical analysis:

At the end of P.Exp.P, three hens of each group were sacrificied by slaughter. Blood samples were collected into heparinized tubes and centrifuged at 2500 rpm for 15 minutes. Plasma samples were then taken, separated into plastic tubes and stored at -20°C until the total plasma protein (TPP), albumin (Alb), total calcium (TC), total phosphorus (TP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were assayed. These parameters were assayed by a colorimetric method using the commercial kits. A portion of heparinized blood from each sample was freshly taken to determine the hemoglobin content. Samples of breast muscle and liver were immediatelly dissected and stored at -20°C until lead concentration were assayed. To determine lead content in eggs, three eggs were boiled and then stored at -20°C. The rest of birds (17 hens) of each group were injected by 0.5 ml/hen from 10% washed sheep red blood cells (SRBC) suspended in saline solution. After 7 days of SRBC injection, blood samples were withdrawn from the wing vein of 3 hens of each group and serum samples were then separated into plastic tubes and stored at -20°C until antibody titer was performed.

At the end of N.Exp.P, 3 hens of each group were also sacrificied by slaughter. Blood, lever and breast muscle samples were then collected as well as 3 eggs of each group were reserved to determine all parameters that previously mentioed at the end of P.Exp.P. Egg, muscle and liver samples were thawed, weighted and dried on 80°C for overnight. Dried samples were crushed weighed and ashed on 500°C for 8 hrs using a muffle furance. Ash samples were then digested with 5ml concentrated nitric acid and analyzed by atomic absorption spectophotometery instrument.

Statistical analysis:

Data for all variables were statically analyzed using the general linear models (GLM) procedure of SAS (SAS, Institute, 1985). Means were separated by using Duncan multiple range test (1955).

RESULTS AND DISCUSSION

Plasma Biochemical Traits

Total plasma proteins (TPP):

Birds fed lead-treated diets showed a significant ($P<0.05$) decrease at the end of the positive experimental period (P.Exp.P) (Table 3). This reduction in total plasma proteins (TPP) was increased as the dietary level of lead was increased where TPP concentrations of 250 and 500 ppm pb-treated groups were 5.66 and 4.87 g/dl, respectively. Adding of clay at a rate of 2.5 and 5% to the low and high lead contaminated diets resulted in a nonsignificant elevation in the level of TPP but still lower than that of control birds. This result could partially modulate the lowering effect of lead on TPP concentration. On the other hand, no significant differences in plasma total protein were observed between clay-treated groups and control one (Table 3). Therefore, it is of interest to conclude that the clay supplementation with the control diet had no effect on TPP while its addition to the lead-contaminated diets reduced the lowering effect of lead on TPP. With respect to the effect of lead, our results are agreement with those obtained by Fathi *et al*. (1999) who reported that serum concentration of total protein of chicks fed lead-added diets were reduced. They suggested that the decrease in serum total protein could be attributed to the failure of protein synthesis. In pekin ducks Abou-Zeid *et al*. (2000) reported that TPP was markedly reduced by supplementing 200 or 400 mg lead acetate/kg of their BW. They interpreted the significant decrease in TPP by the retardation of protein synthesis by liver as a result of the observed destructive changes in the hepatocytes.

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Results of plasma albumin and globulin shown in Table (3) reflected the adverse effect of lead on pb-treated hens. As shown in total protein, plasma albumin and globulin numerically reduction was observed in response to dietary lead acetate. After the P.Exp.P, plasma albumin and globulin concentrations in 250 ppm pb-fed group were decreased to about 72.7 and 81.4% of the control value, whereas in case of the higher dose of lead (500 ppm pb) their concentration decreased to about 62.4 and 70.8% to that of the control. Clay inclusion with lead-treated diets caused an improvement in plasma albumin and globulin values. It is very interesting to note that feeding 5% clay alone resulted in an increase in plasma globulin concentration by about 10.3% than that of the control group. Similar results were obtained by Berisha *et al*. (1996) on hypro hens and Abou-Zeid *et al*. (2000) on Pekin ducks.

After the negative experimental period (N.Exp.P), plasma concentrations of total protein, albumin and globulin were go back to their normal range for all experimental groups and the differences between treatments each other and control group become very narrow for the aforesaid parameters (Table 4). It could be concluded that Gimmizah laying hens are able to overcome the negative effect of lead on TPP, albumin and globulin because they were able to reduce liver lead concentration very fast to be similar to the control group as observed in Table 4.

Hemoglobin content (HB):

The effect of dietary lead on hemoglobin content is shown in Table (3). The results showed a significant ($P<0.01$) decrease in hemoglobin content of birds fed diet containing 250 and 500 ppm pb. The control values were higher than those of treated ones by nearly 56.77 and 64.77% for 250 and 500 ppm pb respectively. Also, values of hemoglobin of birds fed diets free of lead and supplemented with clay only were significantly higher than those fed diet containing 250 and 500 lead ppm levels and supplemented or free of additive tested. These results indicate that the supplementing diets contaminated with lead with clay tended to diminsh the negative effects of lead on hemoglobin. Natural clay can absorb toxic products of digestion and decrease the accumulation of toxic substances in tissues (Mc Collum and Galyean, 1983).

Lead is well known for its capacity to cause anemia which may impair oxygen transport capacity and induce abnormal erythrocyte morphology, (Mc Murry *et al*. 1995). The mechanism by which pb causes anemia is by increasing hemolysis (Hasan *et al*., 1967), interfering with hemoglobin synthesis at the enzyme level (Kao and Forbes, 1973) by reacting with hemoglobin directly (Barltrop and Smith, 1972). According to Berisha *et al*. (1994) showed that which appears after poisoning is results of two main effects, the first includes erythrocytes short life spain while the second inhibits erythrocyte delta-aminolevulinic acid dehydratase (ALA-D). The latter inhibition interfers with the porphobilinoge and therefore, disables the heme normal synthesis. Moreover, it has been reported that lead poisoning inhibits haematopoiesis as a result of changes in mitochondria and ribosomes.

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Antibody titer (Am):

The effect of lead and/or clay supplementation on immunity traits of laying hens are presented in Table (3). It is clearly that there was dose-related, suppressed antibody response to SRBC in hens fed contaminated lead diet. The rate of suppression measured by antibody titer values was 12.6 and 22.3% for both low and high doses of lead treatments respectively comparing with control.

However, adding clay at levels of either 2.5 or 5% to lead-added diets improved immunity response (Titer values). On the other hand, using clay alone by levels imployed in this study did not prove to be immunoactivator (treatments H and G, respectively). Concerning lead contaminated diets our findings agree well with those reported by Vodela *et al*. (1997) in broilers. Also, Koller and Kovacic (1974) reported that oral administration of lead acetate at 13.75, 137.5 and 1.375 ppm to mice in the drinking water resulted in significantly reduced number of antibody plaque forming cells to SRBC. In contrary, Morgan *et al*. (1975). Indicated immuno suppressive effect in Japanese quail. The decreased titer against SRBC caused by test levels of lead reflects a suppression of B cell function. Suppression of humoral immune function was observed in rats exposed to lead level of 3.5 mg/kg BW for 35 to 45 days (Faith *et al*., 1979).

Plasma calcium and phosphorus:

The effects of dietary lead the blood calcium concentration are illustrated in Table 3. Birds fed lead-treated diets (250 and 500 ppm) showed a significant ($P<0.05$) decrease at the end of (P.Exp.P). Similar results were obtained by Murray *et al*. (2000) who found that there was a significant ($P<0.01$) decrease in total plasma calcium at 10 wks of age in birds received diets supplemented with pb at levels of 500 to 1000 ppm pb. They suggested that lead may have a direct effect on the mechanism (s) of calcium metabolism. Adding clay to the basal diet containing lead at levels of 2.5% and 5% succeeded to increase the levels of plasma calcium approximately as equal as control and clay group. Adding clay to bird diets in this study is one of decontamination methods to adsorb lead selectively during the digestive process which rendered most of lead unavailable for absorption from the Gastrointinal tract. After the (N.Exp.P) plasma concentration of total calcium was go back to their normal range for all experimental groups (Table 5). On the other hand, plasma phosphorus level was not significantly affected by experimental treatments, Table 3.

GOT, GPT and ALP enzymes:

With respect to liver function, it could be noticed a significant reduction in the level of GOT and GPT of birds fed basal diet containing 250 and 500 ppm pb compared with control ones (Table 5). This result indicates that lead had a deleterious effect on liver function. The results of Madej *et al*. (1988) showed degenerative changes in the liver and brain cells of lead-fed hens. Examination of hepatic cell of lead-treated ducks revealed vacuolation of its cytoplasm, damaged of its mitochondria and fibrosis (Abou-Zeid *et al*., 2000).

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Similar observations were reported by Yamashiro and Bast (1978) who attributed this to hepatocytic necrosis in the broiler chickens. Adding natural clay to the basal diet containing lead or free of lead resulted in a higher level of GOT and GPT compared with groups fed diets containing 250 and 500 ppm pb but the differences were not significant. Therefore, we can suggest that clay could alleviate the lowering effect of lead on both GOT and GPT activity. After the N.Exp.P, the differences between all treatment and control were not significant. This means that Gimmizah laying hens were able to recover themselves very fast where they can eliminate a large amount of the retained pb in the liver as shown in Table 6. In the light of the present data, exposure to lead at levels of 250, or 500 ppm pb was found to be associated with a significant ($P<0.05$) decrease in the activity of the alkaline phosphatase (ALP) when compared with control group (Table 5). The decrease in the level of (ALP) as affected by lead may be traced to a high degradation rate of ALP in the plasma or a reduced rate of the ALP synthesis within the liver or both of them (Abaza and Azza El- Sabai, 1996). Adding 5% clay to 250 and 500 ppm lead contaminated diets diminished the lowering effect of lead on ALP, however the differences between these two groups and control were not significant. Similar trend was obtained with the group of 500 ppm pb plus 2.5% clay.

Lead residues:

Lead residues in egg, liver and muscle are summarized in Table (6). At the end of P.Exp.P, it could be observed that there is a positive relationship between lead supplementation and the amount of lead in eggs, liver and muscles. In other ward, lead residues were increased in a dose dependent manner where the lead residues in eggs, liver and muscles increased as lead supplementation in the diet increased. It is logically to note that the highest level of lead retention occurred within the liver, while the lowest value was in muscles (Table 6). The amount of lead accumulated in the liver was higher than that of the control group by about 11.9 times at the level of 500 mg pb feeding. The corresponding values for muscles and eggs were higher than that of control by about 2.3 and 5.0 times, respectively. Results obtained by Abou-Zeid *et al*. (2000) are on line with ours. They reported that lead accumulation is increased in a dose-dependent manner within different tissues of pekin ducks.

Lead residue in eggs:

Lead concentration in eggs was significantly ($P<0.0001$) increased in a dose dependent (Table 6). A direct relationship was clearly observed between lead residues in eggs and the supplemental level of pb where feeding of 500 ppm pb resulted in an increase in the egg lead content approximatly two times greater than the feeding of 250 ppm pb. These results are agreement with those obtained by Finley *et al*. (1976) and Mazliah *et al*. (1989). They reported that, the ingestion of lead by laying birds resulted in an increase of lead concentrations in eggs. Burger and Gochfeld (1991) and (1993) reported that birds can release heavy metals in their eggs, therefore they measured lead concentrations in whole eggs of birds to evaluate geographic contamination by heavy metals.

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It can be observed that adding clay to lead-supplemented diets induced a reduction in the level of lead accumulation in egg (Table 6).

Lead residue in liver and muscles:

It is clearly observed that the average of lead concentrations in the liver of 500 ppm pb fed hens was higher than that of their muscle tissues by about 5 times (Table 6). This result could be revealed the important role of the liver in retention of pollutants such as lead, subsequently reduced the level of lead in the blood and finally reduced its content in muscles. These results are agreement with those reported by Jeng, *et al*. (1997) in ducks. Same trend has been also reported by many workers with several species of birds. Lead concentration increased by approximately 7 and 12% in the liver of 250 and 500 ppm lead fed groups when compared with control once, while this increase was about 2% in their muscles for both tested lead levels (Table 6). Addition of clay to lead supplemented diet significantly reduced the liver and muscles lead content as compared with lead treated diet. Moreover, the high level of clay (5%) was more effective in reducing lead retention in the previously mentioned tissues. Adding 5% clay to lead-treated diets (250 and 500 ppm) resulted in a significant reduction of lead accumulation that was ranged between 23.8 and 39.6% in the liver, and 37.8 and 43.9% in the muscles respectively. Also, this reduction ranged between 9.9 and 31.3% in the liver and 20.2 and 25.9% in the muscles when 2.5% clay was added to 250 and 500 ppm pb treated diet, respectively. The role of natural zeolites (kind of clay) in reducing heavy metals toxicity or their accumulation in the tissues was reviewed by Mumpton and Fishman (1977). They indicated that the natural zeolites can absorb toxic products of digestion and decrease accumulation of toxic substances in tissues.

Data presented in Table (6) showed that after birds had consumed diets free of added lead or clay for a period of 4-weeks (N.Exp. P), the concentrations of lead in egg, liver and muscles were go back around their normal values and the significant differences between treatment each other and control were disappeared in most cases. Also, it can observed that, there was a non significant decrease in egg and liver content in lead-clay diets as compared with control. It is interesting to note that muscles had the lowest ability to eleminate lead where its concentration in the muscle become higher than egg and liver after the N.Exp.P These results could be traced to the helpful of natural clay in reducing egg and tissues lead contents and preventing the adverse effect of lead by its chelating and adsorbing properties or it may be act to stimulate the lining of the stomach and intestinal tract, to increase the production of antibodies which could then inhibite the onset of interitis (Mumpton and Fishman, 1977).

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J. Agric. Sci. Mansoura Univ., 27 (12), December, 2002

دراسات فسيولوجية وبيوكيميائية على الدجاج البياض المعامل بالرصاص ودور الطفلة الطبيعية فى منع التأثيرات السيئة وتقليل المحتجز من الرصاص

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اجرى هذا البحث لدراسة تأثير تغذية الدجاج البياض على علائق معاملة بعنصر الرصاص (فى صورة خلات رصاص) على الحالة الفسيولوجية والمناعية وبعض مكونات الدم للطائر مع محاولة تقليل التأثيرات السامة ومحتوى البيضة والأنسجة من الرصاص المحتجز باستخدام الطفلة الطبيعية.

فى هذه الدراسة تم توزيع عدد ١٨٠ دجاجة بياضة عمر ٣٨ أسبوع على ٨ معاملات بالإضافة إلى الكنترول بكل معاملة ٢٠ دجاجة كالتالى:-

الكنترول (عليقه بياض)

المعاملة الأولى: كنترول مضاف إليه ٢٥٠ جزء فى المليون خلات رصاص/كجم علف.

المعاملة الثانيــة: كنترول مضاف إليه ٢٥٠ جزء فى المليون خلات رصاص/كجم علف+٢,٥% طفلة.

المعاملة الثالثــة: كنترول مضاف إليه ٢٥٠ جزء فى المليون خلات رصاص/كجم علف+٥% طفلة.

المعاملة الرابعــة: كنترول مضاف إليه ٥٠٠ جزء فى المليون خلات رصاص/كجم علف.

المعاملة الخامسة: كنترول مضاف إليه ٥٠٠ جزء فى المليون خلات رصاص/كجم علف+٢,٥% طفلة.

المعاملة السادسة: كنترول مضاف إليه ٥٠٠ جزء فى المليون خلات رصاص/كجم علف+٥% طفلة.

المعاملة السابعــة: كنترول مضاف إليه ٢,٥% طفلة.

المعاملة الثامنـــة: كنترول مضاف إليه ٥% طفلة.

استمرت الطيور فى التغذية على هذه المعاملات يوميا لمدة أربعة أسابيع وبعد هذه الفترة تم أخذ عينات دم من الطيور لتقدير بعض صفات الدم البيوكيميائية مثل البروتينات الكلية- الألبيومين – الجلوبيولين – محتوى الدم من الهيموجلوبين وكذلك بعض العناصر المعدنية مثل الكالسيوم والفوسفور بالإضافة إلى قياس نشاط بعض الإنزيمات مثل GOT, GPT والأكالين فوسفاتيز (ALP).

كما تم دراسة الحالة المناعية للطيور وذلك عن طريق قياس مستوى الأجسام المناعية المتكونة ضد الأنتيجين المتمثل فى خلايا الدم الحمراء للغنم. وفى نهاية فترة المعاملة تم أخذ عينات من البيض الناتج مع ذبح ٣ دجاجات من كل معاملة لتقدير الرصاص المحتجز فى كل من البيضة والكبد واللحم. بعد إيقاف المعاملات السابقة لمدة أربعة أسابيع أعيدت نفس القياسات السابقة للتعرف على مدى قدرة الطائر على التخلص من التأثيرات الضارة بمرور الوقت.

- **أظهرت النتائج أن:-**

1) المعاملة بالرصاص سواء بالجرعة المنخفضة (٢٥٠ جزء فى المليون/كجم علف) أو الجرعة المضاعفة (٥٠٠ جزء فى المليون/كجم علف) أدت إلى حدوث انخفاض معنوى فى مستوى كل من البروتينات الكلية ومحتوى الدم من الهيموجلوبين والكالسيوم بالإضافة إلى حدوث انخفاض ملحوظ فى نشاط أنزيمات GOT, GPT والألكالين فوسفاتيز (ALP) كذلك حدث انخفاض ولكن بدرجة غير معنوية بالنسبة لكل من الألبيومين والجلوبيولين وعموما كان مقدار الانخفاض فى التقديرات السابقة مرتبطا بمستوى الرصاص فى العليقة حيث أزداد التأثير السيئ بزيادة مستوى الرصاص.
2) أدى إضافة الرصاص لعليقه الدجاج البياض إلى خفض مستوى الأجسام المناعية المتكونة للمعاملات المختلفة مقارنة بمجموعة الكنترول. وقد وجد أن إضافة الطفلة للعلائق المعاملة بالرصاص أدى إلى رفع مستوى الأجسام المناعية حتى تساوت بمجموعة الكنترول.
3) إضافة الطفلة للعلائق المعاملة بالرصاص أدى إلى تحسن ملحوظ فى قيم التقديرات السابقة وخفض التأثيرات السيئة للرصاص, أما إضافة الطفلة بمفردها فلم يكن لها تأثير ملحوظ فى معظم الحالات.
4) فيما يتعلق بتركيز الرصاص المحتجز فى البيضة والكبد والعضلات, أظهرت النتائج أن قدرة أنسجة الكبد على احتجاز الرصاص كانت تعادل ثلاثة أضعاف المحتجز منه فى البيضة, وأن أنسجة العضلات كانت أقلها احتفاظا بالرصاص, ولوحظ أن كمية الرصاص المحتجزة كانت متناسبة طرديا مع مستواه فى العليقة, وأن إضافة الطفلة للعلائق المعاملة بالرصاص أدى إلى حدوث انخفاض معنوى فى الكمية المحتجزة منه.
5) بعد أربعة أسابيع من إيقاف المعاملات لوحظ تلاشى الفروق المعنوية بين المعاملات بالنسبة لجميع التقديرات السابقة وأصبحت تقديرات الدم تدور حول معدلها الطبيعى.
6) لوحظ أن أنسجة العضلات كانت أقل قدرة من نسيج الكبد والبيضة على التخلص من الرصاص المحتجز بها بمرور الوقت حيث أنها احتوت على تركيز أعلى منه فى نهاية الفترة الثانية.

- ومن خلال النتائج السابق الحصول عليها من هذه الدراسة يمكن التوصية بإضافة الطفلة الطبيعية بمستوى ٥% إلى علائق الدجاج البياض المعاملة بالرصاص وذلك لمنع التأثير السيئ للرصاص .

Table (3). Total plasma proteins, calcium, phosphorus, hemoglobin and antibody titer of Gimizah laying hens fed different levels of clay and/or lead at the first of P.Exp.P[1].

Treatments	Total protein[2] (g/dl)	Albumin[3] (g/dl)	Globulin[3] (g/dl)	Calcium[2] (mg/dl)	Phosphorus[3] (mg/dl)	Hemoglobin[2] (mg/dl)	Antibody[2] Titer
Control (Con)	7.35[a]	3.96	3.39	24.02[a]	6.71	8.93[a]	10.3[ab]
(A)Con+250 ppm pb	5.66[ab]	2.88	2.76	17.65[b]	6.23	3.86[de]	9.0[bc]
(B)Con+250ppm pb+2.5% clay	6.46[ab]	3.45	3.01	21.52[ab]	5.80	4.06[de]	9.5[ab]
(C)Con+250ppm pb+5% clay	6.73[ab]	3.75	2.98	21.70[ab]	6.03	4.30[de]	10.0[ab]
(D)Con+500 ppm pb	4.87[b]	2.47	2.40	17.35[b]	5.80	3.19[e]	8.0[c]
(E)Con+500ppm pb+2.5% clay	5.89[ab]	2.87	3.02	20.18[ab]	5.77	4.50[cde]	10.0[ab]
(F)Con+500ppm pb+5% clay	6.29[ab]	3.39	2.90	21.89[ab]	6.81	4.73[cd]	10.0[ab]
(G)Con+2.5% clay	7.0[a]	3.68	3.32	24.18[a]	6.36	5.83[b]	10.0[ab]
(H)Con+5% clay	7.19[a]	3.45	3.74	24.68[a]	6.86	6.43[b]	10.6 [a]
SE	± 0.61	± 0.50	± 0.57	± 1.72	± 0.54	± 0.46	0.47

[1] P.Exp.P. means the positive experimental period.
[2] Means with different superscripts for treatments and each variable are significantly ($P \leq 0.05$) different.
[3] No significancy was detected between treatments and each variable.

Table (4). Total plasma proteins, albumin, globulin, calcium and phosphorus of Gimizah laying hens fed different levels of clay and/or lead at the end of N.Exp.P[1].

Treatments	Total protein[2] (g/dl)	Albumin[2] (g/dl)	Globulin[2] (g/dl)	Calcium[2] (mg/dl)	Phosphorus[2] (mg/dl)
Control (Con)	6.46	3.29	3.17	24.59	6.93
(A)Con+250ppm pb	6.35	3.88	2.47	20.60	5.76
(B)Con+250ppm pb+2.5% clay	6.60	3.99	2.61	24.02	5.29
(C)Con+250ppm pb+5% clay	6.59	3.41	3.18	21.61	6.40
(D)Con+500ppm pb	6.64	3.18	3.46	22.15	5.95
(E)Con+500ppm pb+2.5% clay	6.20	3.10	3.10	22.01	6.96
(F)Con+500ppm pb+5% clay	6.88	3.33	3.55	20.88	6.74
(G)Con+2.5% clay	6.61	3.11	3.50	24.85	5.90
(H)Con+5% clay	6.81	3.48	3.33	24.17	6.88
SE	± 0.02	± 0.43	± 0.49	± 3.18	± 0.84

[1] **N.Exp.P. means the negative experimental period.**
[2] **No significancy was detected between treatments and each variable.**

Table (5). Levels of GOT, GPT and ALP of Gimizah laying hens fed different levels of clay and/or lead at the end of P.Exp.P[1] and N.Exp.P[1].

Treatments	P.Exp.P			N.Exp.P		
	GOT[2] (µ/100 ml)	GPT[2] (µ/100 ml)	ALP[2]	GOT[2] (µ/100 ml)	GPT[2] (µ/100 ml)	ALP[2]
Control (Con)	303.95[a]	157.25[a]	1275.90[a]	302.01	148.55	1336.18[a]
(A)Con+250 ppm pb	282.74[bc]	141.70[b]	787.35[b]	283.88	143.63	910.09[bc]
(B)Con+250ppm pb+2.5% clay	294.64[abc]	148.98[ab]	744.80[b]	297.85	151.83	935.67[bc]
(C)Con+250ppm pb+5% clay	293.70[abc]	150.69[ab]	1147.30[a]	288.77	151.84	1126.10[abc]
(D)Con+500ppm pb	277.01[c]	144.91[b]	771.78[b]	283.57	150.54	908.70[bc]
(E)Con+500ppm pb+2.5% clay	288.60[abc]	143.84[b]	1083.00[ab]	289.13	144.06	1166.00[ab]
(F)Con+500ppm pb+5% clay	300.50[ab]	148.96[ab]	1188.80[a]	264.24	149.41	1297.50[a]
(G)Con+2.5% clay	292.38[abc]	146.27[b]	775.90[b]	287.57	144.91	879.67[bc]
(H)Con+5% clay	292.75[abc]	145.13[b]	959.17[ab]	310.32	147.41	789.95[c]
SE	± 6.12	3.14	104.20	± 15.13	± 6.32	109.65

[1] **P.Exp.P. and N.Exp.P. means the positive and negative experimental period respectively.**
[2] **Means with different superscripts for treatments and each variable are significantly ($P \leq 0.05$) different.**

Table (6). Lead concentrations in egg, liver and muscles of Gimizah laying hens fed different levels of clay and/or lead at the end of P.Exp.P[1] and N.Exp.P[1].

Treatments	Lead concentration P.Exp.P.			Lead concentration N.Exp.P.		
	Eggs[2] (ppm)	Liver[2] (ppm)	Muscles[2] (ppm)	Eggs[2] (ppm)	Liver[2] (ppm)	Muscles[2] (ppm)
Control (Con)	11.07^{f}	11.65^{f}	11.96^{c}	8.30^{ab}	14.25^{a}	18.00^{a}
(A)Con+250 ppm pb	27.32^{d}	81.01^{c}	21.57^{ab}	3.70^{cd}	12.65^{ab}	10.90^{b}
(B)Con+250ppm pb+2.5% clay	26.97^{d}	72.98^{d}	17.22^{bc}	5.45^{abc}	8.00^{cd}	13.00^{b}
(C)Con+250ppm pb+5% clay	22.43^{e}	61.73^{e}	12.33^{c}	5.20^{bc}	6.35^{d}	11.60^{b}
(D)Con+500 ppm pb	55.33^{a}	139.06^{a}	27.92^{a}	9.10^{a}	12.00^{ab}	10.80^{b}
(E)Con+500ppm pb+2.5% clay	47.74^{b}	95.56^{b}	20.70^{ab}	5.90^{abc}	10.60^{bc}	19.00^{a}
(F)Con+500ppm pb+5% clay	40.77^{c}	85.30^{c}	17.36^{bc}	5.10^{bc}	11.10^{ab}	10.20^{b}
SE	± 1.14	± 0.58	± 58	1.16	0.98	0.91

[1] P.Exp.P. and N.Exp.P. means the positive and negative experimental period respectively.
[2] Means with different superscripts for treatments and each variable are significantly ($P \le 0.05$) different.

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