THE ROLE OF CLAY OR VITAMIN E IN SILVER MONTAZAH LAYER HENS FED ON DIETS CONTAMINATED BY LEAD AT VARIOUS LEVELS.
1-PERFORMANCE AND EGG COMPONENTS.

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SUMMARY

A total number of 240 laying hens and 24 cocks of Silver Montazah strain at 40 weeks of age were randomly divided into 12 groups (20 hens and 2 cocks / each treatment group). Birds in all treatments were nearly similar in the average initial body weights and average daily egg production A 4 x 3 factorial design experiment was performed including four levels of supplemented lead (0. 250,500 and 1000 mg/kg diet) and three levels of feed additives (without clay or vitamin E, 3 % Natural clay, tafla, and 200 mg /kg vitamin E). Live body weight change, egg production and egg weight, egg compenents, fertility and hatchability were studied.

Results obtained reveled that lead contaminated feed caused significant live body weight retardation, lower egg production and egg weight, lower egg component and lower fertility and hatchability. On the other hand, the addition of natural clay or vitamin E to the diets for laying hens caused a significant improve in all aforementioned traits. With respect to interaction between lead and the experimental additives the results obtained showed that, within each lead level clay or vitamin E supplementation recorded higher body weight, increased egg production, egg weight and best egg fertility & hatchability.

On the basis of the results obtained in this study, it can be concluded that the consumption of polluted diets with heavy metal such as lead cause deleterious effects in the productive performance, egg components and reproductive performance of Silver Montazah laying hens, while, dietary addition of natural clay or vitamin E to the diet of laying hens cause beneficial effects on egg components, productive and reproductive performance. Moreover, clay or vitamin E supplementation diminished the toxic effect of lead on all egg quality, productive and reproductive results during treatment period and supported recovering after the lead contamination.

Key words: Lead, growth performance, egg production, fertility and hatchability.

INTRODUCTION

The contaminations of poultry feeds with heavy metals especially lead cause a high reduction in growth rate, feed efficiency and egg production, which result finally great economic loss for poultry farmers. Nearly, all potential food ingredients contain some kinds of heavy metals. Lead is considered one of the major environmental pollutants (Jeng et al., 1997) .

The contamination of laying hen's diets or environment with heavy metals remains a problem for poultry meat industry, food safety regulatory agencies and concerned consumers. According to the extensive use of lead in human activities e.g. industrial processes, plant protection, paint industry and motor-engine emission, the possibilities of bird's diet pollution become more evident.

Previous works on poultry have revealed that lead consumption can cause deleterious affects on growth performance (Youssef et al., 1995 and Abou-Zeid et al., 2000), efficiency of feed utilization (Edens and Melvin, 1989), egg production (Eden and Garlieh, 1983) and reproductive efficiency (Vodela et al., 1997).

Supplementation of poultry diets with natural clays enhances growth rate and egg production. This may be due to improvement in either of feed conversion, digestibility, ability to bind metallic cations and rendering them more available to the bird and nitrogen retention in bird body, in addition to retard the absorption of toxic products of digestion that reduce toxicity. Ability of clays to diminish the harmful effects of radiation may have a role in this respect (Ayyat and Marai, 1997).

Vitamin E has a number of different biological function. One of the most important functions is the role as an intracellular antioxidant. In this capacity Gore and Qureshi (1997) speculated that vitamin E prevents oxidation of unsaturated lipid materials within cells, thus protecting the cell membrane from oxidative damage. Vitamin E sometimes called antisterility vitamin. As reported by Kling and Scares (1980), Japanes quail maintained on a low vitamin E diet for 35 weeks, showed a lower presence of fertile eggs after 20 weeks and hatchability severely depressed probably due to the inadequate deposition of vitamin E in the egg to support embryonic growth

Therefore, the present study was carried out to investigate the efficacy of clay and vitamin E to alleviate severity of lead contamination and its effect on the productive performance, egg quality and reproductive performance of silver Montaza layers.

MATERIALS AND METHODS

This work was carried out at Inshas Poultry Research Farm belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

A total number of 240 hens and 24 cocks of Silver Montazah strain at 40 weeks of age were randomly divided into 12 groups (20 hens and 2 cocks / each treatment group). Birds in all treatments were nearly similar in the average initial body weights and average daily egg production. A 4x3 factorial design experiment was performed including four levels of supplemented lead (0.250 500 and 1000 mg/kg diet) and three levels of feed additives (without clay or vitamin E, 3 % Natural clay, tafla, and 200 mg /kg vitamin E). Tafla is a desert clay and analyzed as soluble cations and anions (meq/ 100 g dry matter soil) were Ca^{++} 0.75, Mg^{++} 0.25, Na^{+} 0.05, K^{+} 0.10, Cl^{-} 0.55, SO_4 0.30 and HCO_3 0.75. Exchangeable cations (meq/100 g DM soil) were 2.65 and available nutrients (mg/100 g dry matter soil) were P 5.0, K 1.2, Mn 2.4, Zn 0.74, Cu 0.30 and Fe 0.55 mg(Maria *et al* 1996). The composition and calculated chemical analysis of the experimental laying diet are presented in Table 1. The birds were fed the contaminated diets from 40 to 52 weeks of age, while at period from 52 to 56 weeks of age, birds were fed diets without lead addition.

Birds of all experimental groups were housed in suitable pens during the experimental period and were kept in the same conditions. Feed and water were offered *ad libitum* and maintained on 16 hours light per day. Body weight was recorded at the start (40 weeks old) then biweekly intervals there after, during the experimental periods. Feed consumption was recorded biweekly, while egg production rate and egg weight were recorded daily.

At 52 and 56 weeks of age ten eggs from each treatment group were randomly selected to measure egg components. Eggs were individually weighted, broken and albumine, yolk and shell weight were recorded to calculate albumine, yolk and shell percentage values.

During the experimental period eggs were collected from each experimental group, then weighed and stored for one week in refrigerator (at 12 ^{o}C and 70 % RH). Eggs were maintained in room temperature for about 12 hours, before incubation. Only sound and normal eggs were fumigated with formaldhyde gas and set in a forced air incubator. Incubation was carried out under recommended conditions for chicks eggs. At the 14^{th} days of incubation period infertile eggs (those having no visible embryonic development) and dead embryo were removed and recorded. Fertility was estimated at the end of hatching period as number of fertile eggs / number of eggs set. Hatchability of eggs from each experimental group was also estimated as ratio of number of chicks hatched to number of fertile eggs.

The obtained data were statistically analyzed by using 4x3 factorial design by Snedecor and Cochran (1982). according to the following model: $Yijk = \mu + Li + Aj + LAij + eijk$

where, μ = the overall mean, Li = the fixed effect of ith lead level in diets (i = 1,.. 4), Aj = the fixed effect of jth feed additives (j = 1,.. 3), LAij = the interaction between the ith lead level and feed additives and eijk = random error. Significant differences were determined by Duncan's Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Layer performance:

Body weight:

Effect of lead pollution:

Growth inhibition is one sign of overt toxicosis in chickens.In this experiment, significant ($P<0.05$) reduction was shown in live body weight with increasing the lead level in the diet, during the whole experimental period when compared with those fed the control diet without lead contamination (Table 2). At 56 weeks of age, the reduction in growth rate was slightly lower than in the other periods, this may be related to the physiological response of the birds to recover toxicity from the lead.

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). Beyer et al. (1988) reported that birds of different species exposed to different levels of dietry lead exhibited cases of proventriculi impacted with feed, reduced feed consumption, smaller weight and death. The assent results concerning live body weight retardation may be also related to the diminishing in hemoglobin synthesis and can react with cell membranes, This may cause increased permeability of the cells and damage or even death of those cells. Lead can disppace calcium in bone, deposit there and from softer, 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).

Decreased body weight was also observed in Japanese quail hens exposed to dietary lead at 500 mg from hatching through reproduction (Edens et al 1976, Edens and Garlich, 1983).A recent study by Vodela et al., (1997) demonstrated a linear relationship between increasing concentration of the lead in feeding diet and a decreasing body weight of broiler breeder hens. However, many other workers found no growth changes due to lead treatments (Custer et al., 1984; Krishman and Marshall 1988; and Jeng et al., 1997). This could be attributed to different doses of administration, compound nature of lead and / or to the animal species.

Effect of feed additives:

Live body weight did not show any significant improvement by the feed additives (Table 2). Final body weight of hens fed diet supplemented with clay or vitamin E slightly increased than those fed control diet. Karelina (1985) and Gonalez et al. (1996) found that supplementing various sources of natural clay in broiler diets increased body gain and improved feed conversion. On the other hand. Ward et al. (1993) found that growth rate decreased with addition of 0.75% sodium zeolite in chick diets. Larry (2002) indicated that vitamin E supplementation in the diets improved the growth rate.

Interaction between feed additives and lead pollution:

Live body weight was not significantly affected due to the interaction effects between feed additives and lead contamination (Table 2). At any lead level, clay supplementation in hen diets recorded higher body weight than the other experimental groups then those fed diets supplemented with vitamin E.The results show that lead poisoning can be partially reduced by providing supplementaly vitamin E and natural clay, but the interaction of vitamin E remains to be elucidated.In this respect Larry (2002) reported that vitamin E improves cellular immune function, which potentially lowers the risk of infection. Also, Shalaby and Ayyat (1999) reported that the natural clay addition in chicken diets reduced the toxicity effects of the pesticides (profenofos and monocrotophos).

Egg production and egg weight:

Effect of lead pollution:

Egg production was significantly ($P<0.01$ or 0.001) dcreased in hens as the concentration of the lead diets increased at all the experimental periods (Table 3). The presence of lead in the diet could have contributed to impaired egg production through the suppression of calcium metabolism. Dietary lead at 200 mg is known to influence egg production and calcium metabolism (Edens and Garlich, 1983). When lead was added to the diet, highly significant decrease occurred in red blood cell delta amino levulinic acid dehydratase (RBCALAD) activity. The lowered enzyme activity may be related to calcium metabolism and egg production (Stone and Scare 1976). Dcreased egg production was also observed in laying hens exposed to lead concentrations of 200 mg /kg diet for 4 weeks (Edens and Garlich, 1983).

Egg weight significantly ($P<0.001$) affected with lead contamination in hen feeds at 40-44 and 48-52 weeks of age (Table 3). Egg weight increased slightly with increasing lead level in hen diets at 48-52 weeks of age. These findings may be attributed to the decrease in egg production when hens fed on the diets contaminated with lead. Estrogen plays a rol in controlling egg weights vai changes in fat metabolism and oviductal protein synthesis (Whirtehead et al., 1993). A decreased level of estrogen receptors (58%) was reported in a human breast cancer cell line exposed to cadmium (Morales et al., 1994). A decrease in uterine estradiol receptors was also observed in rats exposed to 200 mg of lead in drinking water for 35 d (Wiebe and Barr, 1988). Exposure to low concentration of lead in drinking water for 10 weeks in broiler breeder hers resulted in dcreased egg weights (Vodela et al. 1997).On the contrary, Whisenhunt and Maurice (1981) reported that feeding chicken hens on diet containing 500 mg lead/kg diet showed no significant effects on egg production and egg weight.

Effect of feed additives:

Clay or vitamin E supplementation significantly($P<0.01$ or $P< 0.001$) increased egg production and egg weights comparing to hens fed on a diet without supplementation during the whole experimental periods (Table 3) except egg weight during 52-56 weeks of age. Higher values of both egg production and egg weights were also observed by Abd El-Latif (1999) with Japanese quail birds which received vitamin E (25 and 50 mg / kg diet). This observation could explain the important role of vitamin E in female reproductive function (King and Scares 1980). The beneficial effect of vitamin E for egg production was associated with increased plasma concentration of egg yolk precursors, vitellogenin and very low density lipoprotein (Bollengier et al., 1999). According to Elliot and Edwards (1991) natural zeolite a clinoptilolite-bearing rock material, were found to increase egg weight and albumin weight when it was incorporated in the hen's diet at an inclusion rate of less than 10%.

Interaction between feed additives and lead pollution:

The results obtained revealed that egg production and egg weights were significantly ($P<0.05$ and $P<0.01$) affected by the interaction between feed additives and lead contamination during the whole experimental period, except at 52-56 weeks of age (Table 3). Within any lead level, clay or vitamin E supplementation increased egg production and egg weights when compared with the groups fed diets without feed additives. In general, the results indicated that the use of the cation exchange capability to reduce the uptake, and influence the distribution of these heavy metals in poultry tissues is promising. Evans et al. (1993) concluded that the use of synthetic and natural zeolites limited economic benefit or application for improving the performance and egg shell quality in poultry. On the other hand vitamin E supplementation may have enhanced synthesis of egg yolk precursors, uitelloginin and very low density lipoprotein in the liver by protecting the liver from lipid peroxidation and damage to cell membranes.

Fertility and hatchability:

Effect of lead pollution :

Data in Table 4 showed that increasing lead level in the diets of hens significantly ($P<0.001$) decreased the fertility and hatchability percentages when compared with those fed the control diet without lead contamination. It is clear that increasing the exposing time to lead contamination the harmful effect on the fertility and hatchability increased (Table 4).At 52-56 weeks of age, the effect of lead toxicity on fertility and hatchability reduced, these findings may relate to the ability of birds to recover during a layer period of lead toxicity. The reduction in fertility rates in the present study may be due to impaired semen characteristics in this study. Abaza et al., (1996) demonstrated that the lead cause dysfunction in the reproductive and physiological systems of cockerels. It is manifested in the decrease in semen volume and relative sperm motility. Edens et al. (1976) reported reproductive dysfunction increasing significantly the age of sexual maturity in Japanese quail fed diet contained 10, 100 or 1000 mg lead.While the reduction in hatchability rates may be due to the eggs laid by hens treated with lead contained high proportion of source element (Jeng et al., 1997).The egg components are the main source nutrients for the developing embryo and the newly hatched chicks absorb in their body approximately 6% of the total egg yolk. This amount of yolk usually is consumed during the first days of life after hatching. This might mean that lead will be exist in the metabolism of the developing embryo as well as during the early days of the hatched chick.

The obtained results are in agreement with the findings, De Gennaro (1978) who reported that the lead-treated embryos failed to be hatched and the embryos died before 21-day of incubation. Also, Vodela et al. (1997) indicated that the broilers drinking water containing 6.7 mg lead significantly increased the embryonic mortality (68.84%) compared with the control birds (16.16%).

Effect of feed additives :

Fertility and hatchability percentages were insignificantly increased by the clay or vitamin E supplementation feed in the diets during the whole experimental period, except at 48 - 52 weeks of age the egg fertility and hatchability percentages significantly ($P<0.05$) increased (Table 4).This finding may be related to the improvement of egg quality with feed additives

Interaction between feed additives and lead pollution:

The interaction between feed additives and lead level in fertility and hatchability percentages were not significant during all the experimental periods (Table 4).

Egg components:

Effect of lead pollution:

Data in Table 5 showed significant (P<0.001) increase in albumin percentage and significant (P<0.001) decrease in yolk percentage with increasing lead level in the diet at 52 weeks of age , while egg shell percentage were not significantly affected .

Effect of feed additives :

Egg components percentages were not significantly affected by clay or vitamin E supplementation at 52 weeks of age (Table 5). According to Yannakopoylps et al., (1998) natural zeolite was found to increase both egg weight and albumin weight, while yolk weight was not significantly affected. They also found that the yolk : albumin ratio was lower (more albumin) in eggs laid by hens on zeolite treatments . Hossain and Sergio (1995) found insignificant effect of vitamin E on egg quality in broiler breeders.

Interaction between feed additives and lead pollution:

There was no significant interaction between the feed additives and lead level in egg components percentages at 52 weeks of age (Table 5).

On the basis of the results obtained in this study, it can be concluded that the consumption of polluted diets with heavy metal such as lead cause deleterious effects in the productive performance, egg components and reproductive performance of Silver Montazah laying hens, while, dietary addition of natural clay or vitamin E to the diet of laying hens cause beneficial effects on egg components, productive and reproductive performance. Moreover, clay or vitamin E supplementation diminished the toxic effect of lead on all egg quality, productive and reproductive results during treatment period and supported recovering after the lead contamination.

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Table 1. Composition and calculated analysis of the experimental laying diet:

Ingredients	**%**
Yellow corn	66.55
Soybean meal (44 %)	18.00
Wheat bran	5.80
Limestone	6.85
Bone meal	2.20
Premix *	0.30
Sodium chloride	0.30
Total	100.00
Calculated analysis according to NRC (1994):	
ME, kcal / kg	2706
CP %	14.96
Methionine + cystine %	0.49
Lysine %	0.72
Calcium %	3.34
Total phosphorus %	0.65

- Each kg of premix contain : 5, 000, 000 I. U. Vit. A; 1, 250, 000 I. U. Vit. D_3; 2 g Vit. K; 3g Vit B_2 ; 15 g nicotinic acid; 4 g calcium D-Pantothenate; 8 g Vit. B_{12} ; 150 choline chloride ; 80 g D. O. T. (35 Dinitro ortho toluamide); 40 g manganese; 20 g iron; 20 g zinc; 1 g copper; 1 g iodine and 1 g cobalt.

Table 2. Live body weight , g, mean ± SE, of Silver Montazah hens as affected by lead, feed additives and their interaction at different experimental ages.

	Age in weeks				
Items	**40**	**44**	**48**	**52**	**56**
Lead levels (mg/kg diet):					
0	1538.8±28.1	1614.4±28.4	1695.9±28.2	1781.2±27.8[a]	1867.4±30.2[a]
250	1528.4±20.3	1598.9±21.2	1656.1±21.7	1705.7±22.0[ab]	1779.6±26.6[b]
500	1532.4±26.5	1595.2±26.6	1645.7±26.9	1686.4±27.2[b]	1760.9±32.2[b]
1000	1535.3±28.1	1589.2±28.2	1633.7±28.5	1663.4±28.7[b]	1739.2±33.0[b]
Feed additives:					
Without	1529.6±22.3	1589.1±22.5	1644.1±22.8	1693.2±23.1	1772.0±26.7
Clay	1534.7±22.6	1605.8±22.9	1668.5±23.4	1722.4±24.0	1799.8±27.6
Vitamin E	1536.4±21.8	1603.2±22.3	1661.1±22.5	1715.9±23.0	1789.7±26.6
Significance :					
Lead levels (L)	NS	NS	NS	*	*
Feed additives (F)	NS	NS	NS	NS	NS
L x F	NS	NS	NS	NS	NS

Means in the same column within each classification with different letters, differ significantly (P<0.05).
NS = not significant, * P<0.05

Table 3. Egg production (mean ± SE) of Silver Montazah laying hens as affected by lead, feed additives and their interaction during the different experimental periods.

Treatment	Egg production (% / hen / day)				Egg weight (g)			
	40 – 44 weeks	44 – 48 weeks	48 – 52 weeks	52 – 56 weeks	40 – 44 weeks	44 – 48 weeks	48 – 52 weeks	52 – 56 weeks
Lead levels (mg/kg diet)								
0	34.90 ± 0.96[a]	37.09 ± 1.18[a]	49.77 ± 1.29[a]	52.36 ± 1.05[a]	51.24 ± 0.27[a]	50.55 ± 0.27	50.21 ± 0.20[b]	49.98 ± 0.14
250	32.68 ± 1.05[a]	33.32 ± 1.22[b]	47.33 ± 1.21[a]	46.87 ± 1.30[b]	49.72 ± 0.29[b]	50.09 ± 0.37	49.78 ± 0.23[b]	50.45 ± 0.54
500	26.51 ± 1.23[b]	34.65 ± 1.07[b]	41.91 ± 1.24[b]	48.30 ± 1.05[b]	49.40 ± 0.32[b]	50.27 ± 0.31	51.57 ± 0.37[a]	50.26 ± 0.16
1000	23.14 ± 1.17[c]	22.32 ± 1.34[ac]	32.23 ± 1.55[c]	47.02 ± 1.43[b]	49.90 ± 0.31[b]	50.83 ± 0.43	51.94 ± 0.30[a]	50.78 ± 0.30
Feed additives:								
Without	24.43 ± 0.95[a]	28.43 ± 1.13[a]	37.62 ± 1.27[a]	45.66 ± 1.03[a]	50.16 ± 0.27[a]	49.92 ± 0.30[a]	50.28 ± 0.15[a]	50.08 ± 0.38
Clay	33.26 ± 1.04[b]	33.25 ± 1.22[b]	45.30 ± 1.34[b]	50.23 ± 1.18[b]	50.68 ± 0.22[a]	50.58 ± 0.33[a]	51.33 ± 0.22[b]	50.45 ± 0.23
Vitamin E	30.29 ± 1.00[c]	33.83 ± 1.09[b]	45.50 ± 1.19[b]	50.02 ± 0.49[b]	49.36 ± 0.28[b]	50.80 ± 0.28[a]	51.10 ± 0.36[b]	50.57 ± 0.21
Significance:								
Lead levels (L)	***	***	***	**	***	NS	***	NS
Feed additives (F)	***	***	***	**	***	**	***	NS
L x F	**	*	**	NS	***	**	***	NS

Means in the same column each classification with different letters, differ significantly (P<0.05).
NS = not significant, * P<0.05, ** P<0.01 and *** P<0.001.

Table 4. Fertility and hatchability (mean ± SE) of Silver Montazah laying hens as affected by lead, feed additives and their interaction during the different experimental periods.

Treatment	Fertility %				Hatchability %			
	40 – 44 weeks	**44 – 48 weeks**	**48 – 52 weeks**	**52 – 56 weeks**	**40 – 44 weeks**	**44 – 48 weeks**	**48 – 52 weeks**	**52 – 56 weeks**
Lead levels (mg/kg diet)								
0	80.67 ± 0.68[a]	80.71 ± 0.90[a]	82.16 ± 0.42[a]	82.71 ± 0.41[a]	67.06 ± 0.86[a]	67.87 ± 0.64[a]	82.16 ± 0.42[b]	73.39 ± 0.35[a]
250	76.78 ± 0.85[a]	74.49 ± 0.62[b]	73.40 ± 1.87[b]	79.54 ± 0.71[b]	60.83 ± 1.16[b]	59.86 ± 0.71[b]	73.40 ± 1.87[b]	68.79 ± 1.01[b]
500	72.68 ± 1.20[b]	70.59 ± 1.03[c]	68.01 ± 0.93[c]	78.51 ± 0.88[b]	59.32 ± 1.39[b]	56.72 ± 1.16[c]	68.01 ± 0.93[b]	68.48 ± 1.59[b]
1000	65.23 ± 1.93[c]	61.77 ± 0.62[d]	59.04 ± 1.42[d]	75.24 ± 1.74[c]	54.67 ± 1.47[c]	52.79 ± 0.88[d]	59.04 ± 1.42[ad]	63.60 ± 1.41[c]
Feed additives:								
Without	72.75 ± 2.07	70.99 ± 2.23	68.37 ± 3.03[b]	77.55 ± 1.41	59.30 ± 1.85	58.51 ± 2.02	68.37 ± 3.03[a]	67.40 ± 1.87
Clay	74.91 ± 1.77	72.13 ± 2.23	70.75 ± 2.37[ab]	79.35 ± 0.76	61.49 ± 1.67	60.37 ± 1.60	70.75 ± 2.37[ab]	68.98 ± 1.27
Vitamin E	73.87 ± 2.18	72.55 ± 20.7	72.83 ± 2.66[d]	80.11 ± 1.23	60.62 ± 1.51	59.06 ± 1.80	72.83 ± 2.66[b]	69.31 ± 1.03
Significance:								
Lead levels (L)	***	***	***	***	***	***	***	***
Feed additives (F)	NS	NS	*	NS	NS	NS	*	NS
L x F	NS	NS	NS	NS	NS	NS	NS	NS

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

NS = not significant, * P<0.05 and *** P<0.001.

Table 5. Egg components (mean ± SE) of Silver Montazah laying hens as affected by lead, feed additives and their interaction at 52 weeks of age.

Treatment	Egg weight(g)	Shell (%)	Albumin (%)	Yolk (%)
Lead levels (mg/kg diet)				
0	50.21 ± 0.20 b	11.11 ± 0.21	55.85 ± 0.58 [a]	33.04 ± 0.41 [a]
250	49.78 ± 0.23 b	11.79 ± 0.29	57.59 ± 0.50 [b]	30.62 ± 0.52 [bc]
500	51.57 ± 0.37 a	11.28 ± 0.26	57.23 ± 0.66 [b]	31.49 ± 0.56 [b]
1000	51.94 ± 0.30 a	11.11 ± 0.24	59.94 ± 0.80 [c]	28.93 ± 0.70 [c]
Feed additives:				
Without	50.28 ± 0.15 a	11.32 ± 0.19	57.82 ± 0.41	30.86 ± 0.39 [a]
Clay	51.33 ± 0.22 b	11.22 ± 0.21	57.81 ± 0.65	31.46 ± 0.59 [a]
Vitamin E	51.10 ± 0.36 b	11.42 ± 0.23	57.82 ± 0.69	30.75 ± 0.60 [a]
Significance:				
Lead levels (L)	***	NS	***	***
Feed additives (F)	***	NS	NS	***
L x F	***	NS	NS	NS

Means in the same column within each classification with different letters, differ significantly (P<0.05).
NS = not significant and *** P<0.001.