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THE INFLUENCE OF LEAD ON EGG PRODUCTION AND CALCIUM METABOLISM IN LAYERS AND NEWLY HATCHED CHICKENS

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Shaver hybrid laying hens at the beginning of laying were supplied feed with added lead at a dose of 25 or 500 mg/kg for 60 days. The control group of layers was fed a complete mixed feed for laying hens containing 174g/kg crude protein, 11.8 MJ/kg ME, 2.96% calcium, 0.53% phosphorus, 45 mg zinc, 46 mg manganese and 5.2 mg/kg lead.

The addition of lead to the feed for layers at a dose of 500 mg/kg decreased the number of erythrocytes, level of haemoglobin, serum concentration of calcium, weight of eggs and the quality of eggshells. The activity of serum alkaline phosphatase was higher in comparison with the control. The chickens hatched from eggs laid by these layers showed a significant decrease in serum calcium and alanine transferase levels and in the percentage of ash in tibia. Neither clinical nor pathological-morphological changes were observed. The addition of lead at a dose of 25 mg/kg feed caused no significant changes in the investigated health and egg production parameters.

Key words: layers, chickens, lead, eggshell quality, haematological changes, calcium, bones.

INTRODUCTION

Lead, as a cumulative poison, interferes with various functions of an organism at the subcellular level (Chisolm *et al.*, 1974). In the cell nucleus it causes inclusions, polyploidy, abnormal mitoses and membrane damage (Lake and Gerschenson, 1978, Dubreuil *et al.*, 1979). It disturbs phosphorylation and ionic transport in mitochondria (Pincus and Saccar, 1979). The intracellular metabolism of lead overlaps with that of calcium resulting in interactions between these two elements and disturbances in ionic transport (Gordon *et al.*, 1979). Lead causes damage to the nervous (Needelman *et al.*, 1990), haemopoietic, gastrointestinal, urinary and reproductive systems (Goyer, 1995).

Lead affects haem synthesis through inhibition of the enzymes delta-aminolevulic acid dehydrogenase, haem synthetase, pyrimidine-5-nucleotidase, as well as uroporphyrin and coproporphyrin decarboxylase (Bakalli *et al.*, 1990, 1995, Berisha *et al.*, 1994, Osweiler, 1996). Lead causes reversible renal tubular dysfunction with cell atrophy and glomerular sclerosis (Goyer, 1971, Goyer *et al.*, 1989).

The intracellular metabolism of lead overlaps mainly with that of calcium (Gordon *et al.*, 1979). Thus, Bauman *et al.* (1994) observed interactions between vitamin D_3, calcium and lead and found that vitamin D increases accumulation of lead in the osseous tissue and decreases its mineralization. Fullmer (1997) observed interactions between 1,25 dihydroxy-vitamin D, calcium-binding protein and the concentration of calcium and lead in the blood. A low concentration of calcium in the feed increases production of 1,25 $(OH)_2D$ and calcium-binding protein, which has an affinity also for lead and thus increases its retention in the organism. Lead intoxication and resulting damage to the kidneys can disturb the production of 1,25 $(OH)_2D$ and thus also calcium absorption in the gut (Edelstein *et al.*, 1984).

In the case of chronic intoxication lead accumulates predominantly in bones. Finlei and Dieter (1979) observed a higher content of lead in bones with a higher proportion of bone marrow. According to Osweiler (1996), the primary deposition of lead in bones takes place in the zone of active growth. Nitsevich (1988) observed that intoxication with lead due to its presence in eggs at a level of about 15 mg/100g resulted in decreased activity of osteoblasts in the femoral bones of chicken embryos. The influence of lead on metabolism of calcium and its content in bone marrow can result in poorer quality of eggshells (Cristea *et al.*, 1970). Lead passes to the eggshells and yolks and its level in the blood of layers decreases (Mazliah *et al.*, 1989). Thus, lead can play an important role in reducing the quality of eggshells and bones and depressing the growth of chicks.

MATERIAL AND METHODS

The experiment was carried on laying hens of the Shaver hybrid, in the first month of laying. The layers were reared on deep litter at identical conditions with regard to 14-h daylight regimen, environmental temperature and humidity. For 7 days before the experiment they were allowed to acclimatize to the experimental conditions.

The control group of layers was fed a commercial complete mixed feed for laying hens.

Table 1: Composition of feed

Ingredient	Amount %	Chemical composition	
Soybean meal	13.5	N-compounds	174 g/kg
Maize meal	35.0	ME	11.8 MJ/kg
Wheat meal	35.0	Ash	150 g/kg
Fodder wheat flour	1.8	Crude fibre	60 g/kg
Fish meal	1.5	Lysine	7.0 g/kg
Meat-bone meal	2.0	Methionine	3.5 g/kg
Yeast (Vitex)	1.0	Calcium	2.6 %
Mineral supplements	8.8	Phosphorus	0.53 %
Feed additives premix	1.4	Zinc	45.0 g/kg
		Manganes	46.0 g/kg
		Lead	5.2 g/kg

The first experimental group of layers received feed which contained lead at a dose of 25 mg/kg. The second experimental group was fed rations containing 500 mg/kg feed. Lead was added in the form of lead acetate. Laying hens of both experimental groups received the same mixed feed as the control layers. Each group consisted of 10 layers which were supplied the specified feed for 60 days.

Starting from day 30 of the experiment, eggs laid by all three groups were incubated and the chickens hatched from them were examined on day 1 of age. The blood for biochemical examination was obtained by decapitation of chicks and hens at the end of the experiment. Biochemical examinations in blood sera were carried out photometrically using Lachema test kits (Brno, Czech Republic). Haematological examinations were conducted according to the methods described by Slanina et al. (1985). The blood for haematological examination was withdrawn from v. cutanea ulnaris. Tibia of one-day-old chickens were extracted to remove fat and heated in a muffle furnace to determine the percentage of ash. The layers were observed for clinical symptoms and clinical and pathological-morphological examinations were carried out at the end of the experiment. The results were evaluated by Student's t-test and the significance of differences between control and experimental groups was determined.

RESULTS

Clinical examination showed no changes in the health of control and experimental layers. Similarly, post-mortem and histological examination of specimens from liver, kidneys, jejunum, aorta, cerebellum and brain cortex showed neither pathological changes nor differences between experimental and control layers.

The addition of lead had an adverse effect on egg production (Table 2). The weight of the eggs decreased from 59.7 g to 58 g in the layers from the 1st experimental group and to 55.9 g in those from the 2nd experimental group ($P<0.005$) in comparison with the control. The 2nd experimental group showed also a significant decrease ($P<0.005$) in the strength (28.2 N) and thickness of eggshells (0.369 mm) in comparison with the control layers (32.8 N, 0.397 mm, resp.) and the layers from the 1st experimental group (31.8 N, 0.386 mm, resp.). These changes were manifested in the percentage of eggs with damaged eggshells which was 5.6 % in the control and 8.4 % and 14.4% in the 1st and 2nd experimental groups.

Haematological examinations (Table 3) revealed a significant decrease ($P<0.05$) in the number of erythrocytes in the layers receiving feed contaminated with 500 mg lead/kg feed (2.5 T/l) in comparison with the control layers (3.29 T/l). The layers which received feed contaminated with 25 mg lead/kg feed exhibited an insignificantly lower (3.01 T/l) number of erythrocytes in comparison with the control. Layers of the 2nd experimental group also showed significant ($P<0.05$) decrease in haemoglobin level (86.2 g/l) in comparison with the control (97.6 g/l) and with the 1st experimental group (96.9 g/l). The number of leukocytes differed insignificantly between the groups of layers (35.8; 31.2 and 29.4 G/l respectively).

Table 2. The influence of lead on egg production

Groups		Control	Addition of lead to the feed	
			25 mg/kg	500 mg/kg
Egg weight	x	59.7	58.0	55.9
g	n	39.0	35.0	28.0
	SD	2.92	3.44	3.78
	P		-	0.005/0.05
Eggshell strength	x	32.8	31.8	28.2
N	n	39.0	33.0	25.0
	SD	2.54	2.95	3.07
	P		-	0.005/0.005
Eggshell thickness	x	0.397	0.386	0.369
mm	n	39.0	35.0	28.0
	SD	0.013	0.011	0.017
	P		-	/0.005
Eggs with damaged shells		5.6	8.4	14.4
%				

P - significantly different

Table 3 Haematological examinations of laying hens

Groups		Control	Addition of lead to the feed	
			25 mg/kg	500 mg/kg
Erythrocyte counts	x	3.29	3.01	2.40
T/l	n	10.0	10.0	10.0
	SD	0.442	0.301	0.196
	P		-	0.05/-
Hemoglobin concentration	x	97.6	96.9	86.2
g/l	n	10.0	10.0	10.0
	SD	8.6	9.1	8.8
	P		-	0.05/0.05
Leukocyte counts	x	35.8	31.2	29.4
G/l	n	10.0	10.0	10.0
	SD	4.8	4.6	3.8
	P		-	-

The higher dose of lead affected the blood content of calcium (2.6 mmol/l) in comparison with the control (4.3 mmol/l) and with the layers of the 1st group (4.7 mmol/l, $P<0.01$). The highest content of phosphorus was found in the 2nd experimental group (1.43 mmol/l) which was followed by the control (1.1 mmol/l) and the 1st experimental group (0.92 mmol/l). The differences in blood phosphorus content were not significant. Changes in the blood content were manifested in the activity of alkaline phosphatase. It was significantly higher ($P<0.01$) in the layers receiving feed contaminated with 500 mg lead per kg feed (5.82 μkat/l) in comparison with control layers (3.22 μkat/l) and the layers from the 1st experimental group (3.72 μkat/l). The activity of aspartate aminotransferase reached 2.3 μkat/l in the control, 2.0 μkat/l in the 1st and 1.9 μkat/l in the 2nd experimental group of layers. The activity of alanine aminotransferase in the layers of individual groups was 0.017, 0.018 and 0.022 μkat/l respectively. The differences in the activities of the above mentioned enzymes were not significant (Table 4).

Table 4.: Biochemical examination of blood serum of laying hens

Groups		Control	Addition of lead to the feed	
			25 mg/kg	500 mg/kg
Ca concentration	x	4.3	4.7	2.6
mmol/l	n	10.0	10.0	10.0
	SD	0.69	0.75	0.98
	P		-	0.01/0.01
P concentration	x	1.1	0.92	1.43
mmol/l	n	10.0	10.0	10.0
	SD	0.29	0.24	0.33
	P		-	-
Activity of alkaline phosphatase	x	3.22	3.72	5.82
μkat/l	n	10.0	10.0	10.0
	SD	1.022	1.081	1.901
	P		-	0.01/0.01
Activity of aspartate	x	2.3	2.0	1.9
aminotrasferase	n	10.0	10.0	10.0
μkat/l	SD	0.11	0.09	0.14
	P		-	-
Activity of alanine	x	0.017	0.018	0.022
aminotransferase	n	10.0	10.0	10.0
μkat/l	SD	0.002	0.005	0.004
	P		-	-

When examining the chickens hatched from eggs laid by the hens from each group (Table 5) we observed a significant decrease in the content of calcium in

the 2nd experimental group (1.28 mmol/l) in comparison with the control (1.91 mmol/l, P<0.005) and the 1st experimental group (1.76 mmol/l, P<0.01). The content of ash in chick tibia was 23.5% in the control, 32.8% in the 1st and 30.9% in the 2nd experimental group. The difference between the latter group and the control was significant (P<0.025). We observed no significant differences in the activity of alkaline phosphatase between individual groups of chickens (71.8, 76.0 and 90.1 μkat/l) although the highest activity of this enzyme was found in the 2nd experimental group. The activity of alanine aminotransferase was significantly higher in both the 1st 4.27 μkat/l, P < 0.005) and 2nd experimental group (3.7 μkat/l, P<0.005) in comparison with the control (2.26 ukat/l). The activity of aspartate aminotransferase showed no significant differences between individual groups (2.01, 1.93 and 1.965 μkat/l).

Table 5.: Examination of one day chicks

Groups		Control	Addition of lead to the feed	
			25 mg/kg	500 mg/kg
Ca concentration	x	1.91	1.76	1.28
in the blood serum	n	6.0	6.0	5.0
mmol/l	SD	0.17	0.19	0.14
	P		-	0.005/0.01
Activity of alkaline	x	71.8	76.0	90.1
phosphatase	n	6.0	6.0	5.0
μkat/l	SD	12.6	14.2	21.8
	P		-	-
Activity of aspartate	x	2.01	1.93	1.965
aminotransferase	n	6.0	6.0	5.0
μkat/l	SD	0.07	0.06	0.08
	P		-	-
Activity of alanine	x	2.26	4.27	3.7
aminotransferase	n	6.0	6.0	5.0
μ/l	SD	0.36	0.53	0.42
	P		0.005	0.005/-
Ash in tibia	x	33.5	32.8	30.9
%	n	6.0	6.0	5.0
	SD	1.24	1.73	1.48
	P		-	0.025/-

DISCUSSION

The intensification and industrial character of poultry rearing brings along new diseases and production disorders. This is also related to the appearance of new hybrids with higher egg yields and growth rate but also more susceptible to nutritional disturbances, environmental conditions and action of various toxic

substances. According to Berg *et al.* (1980) contamination of the environment with lead reaches a level which can affect health, growth and productivity also in poultry.

The adverse influence of lead on metabolism of calcium (Gordon *et al.*, 1979) can affect unfavourably the growth of chicks (Morgan *et al.*, 1975, Bakalli, 1995, Sinovec *et al.*, 1999) and the quality of eggshells (Cristea *et al.*, 1970). Our experiment also showed that addition of lead to chicken feed at a dose of 500 mg/kg for 60 days decreased serum calcium levels in layers, increased activity of alkaline phosphatase, decreased the weight of eggs, the quality of eggshells and increased the number of eggs with damaged shells. We did not observe such changes with 25 mg lead per kg of feed except for egg shell thicknes

Approximately 25-65% of calcium present in the bones is used for production of eggshells (Cox, 1972). The decrease in the quality of eggshells with increasing age of layers has been ascribed to lower transformability of the osteoid tissue and decreased supply of calcium from bones of older layers (Petersen, 1965, Schubert and Gruhn, 1975, Šaly *et al.*, 1979). Lead, through its influence on calcium metabolism in bones can be the cause of poor quality eggshells. An adverse influence of lead on the strength and thickness of eggshells was observed also in hens in the first month of laying and we can assume that this effect might be even more intensive in older layers.

According to Cibulka *et al.* (1986), lead at a dose of 300 mg/kg feed can pass to eggs and embryonal organs. The decrease in serum calcium and ash content in tibia of one day old chicks, hatched from eggs of layers which obtained feed contaminated with lead at a dose of 500 mg/kg feed, points to the adverse influence of lead during embryonal development on mineralization of bones and the subsequent growth of chickens.

The incidence of osseous diseases increases together with a decrease in the quality of eggshells which results in considerable economic losses. Bracewell (1982) investigated 11 most frequently occuring disturbances of the locomotive system in poultry and identified that they were caused frequently by the presence of toxins in feed and particularly by combined and additive actions of various factors. Chronic lead intoxication can contribute to such disorders.

The levels of lead tested in our experiment in layers for the period of 60 days produced neither clinical symptoms nor induced pathological-morphological changes detectable at final post-mortem examination. No decrease in blood hemoglobin below the physiological limit was observed. The enzymatic examinations also failed to indicate damage to respective organs. On the basis of their experiments Bafundo *et al.* (1984) concluded that the content of lead at a level of 1100-3300 mg/kg feed is toxic to growing chicks. Bacalli (1995) reported, that lead is toxic to chickens at doses lower than those suspected previously and ascribed its toxicity to a high degree of genetic variation.

Mazliah *et al.* (1989) investigated chronic intoxication with lead and observed that adult hens tolerate higher levels of lead in their blood compared to chickens without showing any clinical symptoms and that lead is eliminated from their organism also through eggs and eggshells. Our experiments indicate an adverse influence of lead on egg production and mineralization of embryonal bones at levels lower than those which can produce clinical and pathological anatomical changes.

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UTICAJ OLOVA NA PROIZVODNJU JAJA I METABOLIZAM KALCIJUMA KOD NOSILJA I IZLEŽENIH PILIĆA

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SADRŽAJ

Nosilje hibrida Shaver su tokom prvih 60 dana nosivosti dobijale hranu sa dodatkom 25 i 500 mg/kg olova. Kontrolna grupa nosilja je hranjena kompletnom krmnom smešom sa 174g/kg sirovih proteina, 11.8 MJ/kg ME, 2.96% kalcijuma, 0.53% fosfora, 45 mg cinka, 46 mg mangana i 5.2 mg/kg olova.

Dodavanje olova u hranu za nosilje u dozi 500 mg/kg imalo je za posledicu smanjenje broja eritrocita, koncentracije hemoglobina, koncentracije kalcijuma, te'ine jaja i kvaliteta ljuske jajeta. Aktivnost serumske alkalne fosfataze je bila veća u poređenju sa kontrolnom grupom. Pilići izlečeni iz jaja poreklom od ovih nosilja su imali značajno manju koncentraciju kalcijuma u serumu, manju aktivnost serumske alanin transferaze i manje pepela u kostima. Kod njih nisu registrovane kliničke i patomorfološke promene. Dodatak olova u dozi od 25 mg/kg nije dovodio do statistički značajnih promena u ispitivanim parametrima kvaliteta jaja.