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Effect of Supplementing Diet with Expellers from Evening Primrose (*Oenothera biennis* L.) upon the Content of Lead in Liver and Kidneys and Its Fecal Elimination After Loading with Lead Acetate in Broiler Chickens

Wpływ dodatku do paszy wytłoczyn z nasion wiesiołka dwuletniego (*Oenothera biennis* L.) na stężenie ołowiu (Pb) w wątrobie i nerkach oraz jego eliminację z kałem u kurcząt brojlerów obciążonych octanem ołowiu

Oleiferous seeds of evening primrose owe their therapeutic and dietetic popularity to indispensable unsaturated fatty acids: linolenic and gamma-linolenic ones (5). These acids are structural components of tissues, building protein-lipid cell membranes (12) and also they constitute precursors of prostaglandins and other eicosanoides – compounds of multidirectional physiological effect (10).

The oil expellers of evening primrose seeds, being the waste after producing medical oil, may by applied as valuable fodder component (8, 9). They still contain about 50% of not extracted oil (3), 15% protein of the composition similar to that of soybean protein 5% carbohydrates and 42% fibre (4).

It was found out that evening primrose expellers, added to diet, decreased significantly the concentration of lead in liver and kidneys of broiler chickens (9) when applied as 20% energy and protein substitute.

The goal of the studies was to estimate the effect of the evening primrose seeds expellers added to the diet as 20% substitute of energy and protein on the content of lead in liver and kidneys and its fecal elimination after loading with lead acetate in broiler chickens.

MATERIAL AND METHODS

The studies were conducted on eight-week-old Astra B2 broilers of mean body mass of 2.5 kg. The chickens were housed in separate cages at 20°C ±1.5°C, with the maintainance of natural, twenty-four-hour lightening system. The birds were ensured free access to water and feeds. The broilers were divided at random into two groups. The first, control group was fed with standard soybean-corn fodder and the experimental one on diet supplemented with the expellers of evening primrose seeds, which constituted 20% of energy and protein substitute (Table 1).

After the fortnight's adaptation to nutritive conditions, all chickens from both groups were given lead acetate *per os* at a single dose of 50 mg/kg body weight, before their morning feeding.

To determine the initial Pb concentration in liver and kidneys before the application of lead acetate, and after loading with $Pb(CH_3COO)_2$, the chickens were decapitated and the organs were collected after the first, fourth and seventh day. Each of the tested groups was composed of 6 specimens, 48 birds altogether.

During the experiment, once a day, feces were collected for the analysis of Pb content. Blood samples were taken to determine the activity of delta-amino-levulin acid dehydratase (ALA-D) and collected into heparinized test-tubes from wing veins of the birds before the application of lead acetate, and then at the 30th, 60th, 90th, 120th and 150th minute, at the 3rd, 4th, 5th, 6th, 8th, 12th and 24th hour as well as on the 2nd, 3rd, 4th, 5th, 6th and 7th day.

Pb concentrations in liver, kidneys, fodder and feces were determned with the spectrophotometric method. The tissues were mineralized with the mixture of nitric and perchloric acids, where fodder and feces, after having been dried, were mineralized into dry mass in a furnace at 460°C. The lead for spectrophotometric determinations was extracted to the organic phase with the Yeager method as adapted by Żmudzki (13).

The activity of ALA-D in blood was determined with the Tomokuni method (11).

The results were statistically analysed with t-Student test.

RESULTS AND DISCUSSION

Mean lead concentration in standard soybean-corn diet was 0.69 μg/g, while in experimental fodder, supplemented with the expellers from evening primrose seeds was 0.54 μg Pb/g.

The initial Pb concentration, in the liver and kidneys of the control group was 0.29 μg Pb/g and 0.12 μg Pb/g fresh tissue, respectively. The concentration of Pb in the liver and the kidney of chickens fed on the diet supplemented with evening primrose expellers (experimental group) was 0.19 μg/g and 0.06 μg/g, respectively. The differences between the control and experimental chickens in livers and kidneys Pb content amounting 34.5% for liver and 51.6% for kidneys were statistically relevant (Table 2).

After having loaded the birds with lead acetate (50 mg/kg body weight), Pb concentration in liver and kidneys increased. After the first 24 hrs, mean Pb concentration in the liver of chickens from the control group was 6.28 μg/g tissue, whereas in the experimental group – 4.23 μg/g of fresh tissue. This difference in Pb concentration of the liver from control and experimental group amounting 32.6% was statistically relevant (Table 2).

In the kidneys of control hens the concentration of Pb after the first 24 hrs from lead acetate loading increased on the average up to 4.07 μg Pb/g tissue, and in the experimental group up to 2.67 μg Pb/g fresh tissue, on the average. The difference of Pb concentrations in the kidneys amounted to 34.4% and was statistically relevant (Table 2). In the next days the amount of Pb in liver and kidneys of the birds lowered gradually, however, at the 7th day its concentration in the liver of the control group was on the average 3.1, and in the experimental

group 3.5 times higher in comparison to initial values obtained from chickens not loaded with lead acetate (Table 2).

In the kidneys of the chickens, which received $Pb(CH_3COO)_2$, the Pb concentration was 2.04 μg/g tissue for a control group after seven days from loading, and for the experimental 1.37 μg/g tissue. The recorded differences were confirmed with the statistical analysis. Mean values of Pb concentrations in the liver and kdney of broiler chickens are presented in Table 2.

Pb concentration in feces of chickens from control group fed on standard fodder was 14.3% higher than the value obtained from chickens fed on diet with evening primrose seed expellers, however, this difference was not confirmed statistically. In the feces from the chickens of the control group loaded with lead acetate, 178.2 μg Pb/g dry mass was found, on the average, after the first 24 hrs. The concentrations of Pb in the feces of the chickens from the experimental group was higher amounting to 196.8 μg/g and it corresponded with 50.8% of the applied Pb in the form of lead acetate.

After the passage of two days, Pb concentration in the feces of the chicken from the control group was 93.3 μg/g on the average, which, after having considered the whole amount of feces, corresponded with 24.1% of the applied dose of lead acetate. In the feces of the chickens from the experimental group Pb concentration was 104.5 μg/g which amounted to 26.9% of the applied dose of lead acetate.

During the first two days, chickens of the control groups excreted about 70.2% of given Pb with feces, and of the experimental group about 77.7%, from the whole dosis of lead acetate. This difference of 7.5% was not statistically confirmed. On the 3rd, 4th, 5th, 6th and 7th day, this difference between two groups increased up to 25% on the average, and this value was statistically relevant (Table 3).

Before the application of lead acetate, the activity of delta-amino-levulin acid dehydratase (ALA-D) in the erythrocytes of control as well as experimental chickens was, on the average, 2.3 μM PBG/h/cm^3 of erythrocytes for both groups. After the application of $Pb(CH_3COO)_2$ the activity of the enzyme began to decrease gradually. At the 60th minute the inhibition of the activity was 4.3%, at the 90th minute 13%, and after 3 hrs the decrease reached 43.4% and it remained at the same level for 18 hrs. It appears from the presented dependence between inhibition degree of ALA-D and Pb concentration (Fig. 1) that 43.4% inhibition of enzyme activity corresponds with about 90 μg Pb/100 cm^3 of blood.

After 24 hrs the activity of ALA-D in the erythrocytes of chickens started to increase and thus inhibition of activity equalled: on the 2nd day 39.1%, on the 3rd day 34.8%, on the 4th day 30.4%, on the 5th day 26.0%, on the 6th day 21.7% and on the 7th day 17.4%. The level of ALA-D activity inhibition in erythrocytes was similar for both tested groups, and the differences did not exceed 10% and were statistically irrelevant (Fig. 2).

Tab. 1. Composition of experimental and control mixture applied during the studies

Kind of raw material	Group	
	control	experimental
Primrose seeds expellers	–	20.00
Extracted soybean meal	35.00	27.50
Corn meal	53.00	40.50
Fish meal	5.00	5.00
Soybean oil	3.00	3.00
Fodder chalk	1.40	1.40
Fodder phosphate	1.30	1.30
Fodder salt	0.30	0.30
Polfamix DKA	1.00	1.00
Total protein in %	23.90	23.90
EM (MJ/KG)	12.35	12.80

Tab. 2. Lead concentration in liver and kidneys of chickens ($\bar{x} \pm s$)

Time of sampling (days)	Group			
	control		experimental	
	liver	kidneys	liver	kidneys
Before Pb loading	0.29 ±0.06	0.12 ±0.04	0.19 ±0.05	0.06 ±0.02
1	6.28 ±1.81	4.07 ±1.76	4.23 ±1.74	2.67 ±1.23
4	3.71 ±0.97	3.33 ±0.92	2.42 ±0.76	2.00 ±0.66
7	0.91 ±0.45	2.04 ±0.51	0.67 ±0.41	1.37 ±0.50

Results were presented in μg Pb/g of fresh tissue, number of chickens in group – 6, all differences between groups significant at $p \leqslant 0.05$.

Tab. 3. Lead concentration in chickens feces ($\bar{x} \pm s$)

Times of feces collection (days)	Group	
	control	experimental
Before Pb loading	0.42 ±0.16	0.36 ±0.12
1	178.20 ±36.5	196.80 ±37.7
2	93.30 ±13.2	104.50 ±16.4
3	48.60 ±9.6*	36.00 ±6.4*
4	33.60 ±6.1*	25.20 ±4.7*
5	19.20 ±2.8*	15.30 ±2.6*
6	9.00 ±2.4*	6.60 ±2.4*
7	5.10 ±2.2	3.60 ±2.0

Results were given in μg Pb/g of dried feces, number of chickens in group – 6, * – significance at $p \leqslant 0.05$.

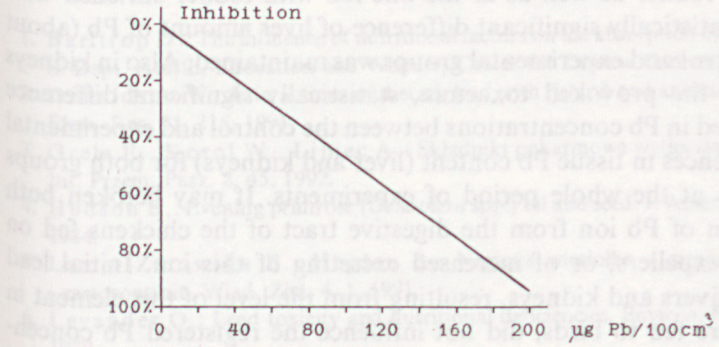

Fig. 1. Dependence of the degree of ALA-D activity inhibition in erythrocytes upon lead concentration in hen blood

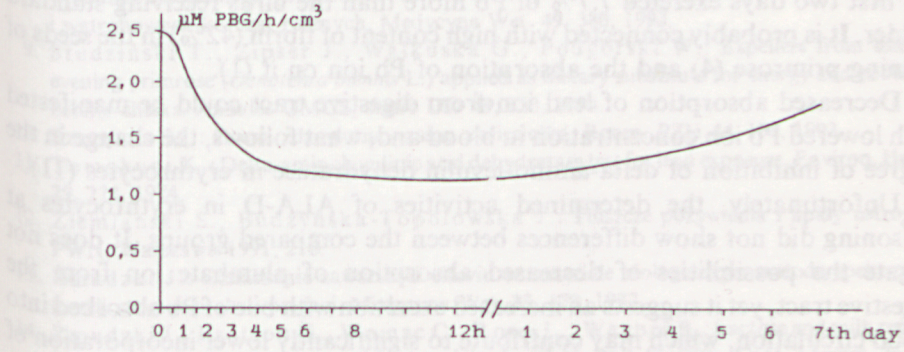

Fig. 2. Changes in ALA-D activity in erythrocytes after the application of 50 mg $Pb(CH_3COO)_2$/kg body mass of a hen. Note: ALAD-D – delta-amino-levulin dehydratase; PGB – porphylinogene (product of reaction)

It was proved that the amount of Pb which can be absorbed from the digestive tract to tissues and organs is dependent upon the dietary ingrediens (1, 6, 14), among which chelating substances as phytate seem to be the most important (7). On the other hand, enhanced Pb toxicity itself for the organism depends upon the influence of the type of consumed fats, and the content of saturated and unsaturated fatty acids in them (2). The observation seems to be relevant, according to which broilers revealed significantly lower levels of Pb in livers and kidneys, despite being fed on fodder enriched with evening primrose seeds expellers, still comprising marked amounts of fat. Probably other constitutes of the applied experimental diet and among them perhaps phytochelates may be responsible for stated differences (7).

Loading chickens with lead acetate caused more than twentyfold increase of load amounts in the livers of the chickens after the first 24 hrs, both in the group

fed with standard fodder as well as in the one fed with fodder enriched with expellers, yet its statistically significant difference of liver amount of Pb (about 32%) between control and experimental groups was maintained. Also in kidneys after 24 hrs from the provoked toxaemia, statistically significant difference (34%) was preserved in Pb concentrations between the control and experimental groups. The differences in tissue Pb content (liver and kidneys) for both groups reached 26%-51% at the whole period of experiments. It may betoken both lowered absorption of Pb ion from the digestive tract of the chickens fed on evening primrose expellers, or of increased excreting of this ion. Initial lead concentrations in livers and kidneys, resulting from the level of this element in fodders, which were fed to birds, did not influence the registered Pb concentration after poisoning.

The analysis of feces excreted after poisoning showed that broilers fed with fodder enriched with 20% supplement of evening primrose seeds expellers during the first two days excreted 7.7% of Pb more than the birds receiving standard fodder. It is probably connected with high content of fibrin (42%) in the seeds of evening primrose (4) and the absorption of Pb ion on it (1).

Decreased absorption of lead ion from digestive tract could be manifested with lowered Pb ion concentration in blood and, what follows, the change in the degree of inhibition of delta-amino-levulin dehydratase in erythrocytes (11).

Unfortunately, the determined activities of ALA-D in erythrocytes at poisoning did not show differences between the compared groups. It does not negate the possibilities of decreased absorption of plumbate ion from the digestive tract, yet it suggests an increased excretion with bile of Pb absorbed into blood circulation, which may contribute to significantly lower incorporation of this element into tissues.

CONCLUSIONS

1. Feeding chickens on diet supplemented with evening primrose seeds expellers as 20% substitute of energy and protein lowers the level of Pb in liver and kidneys of birds.

2. Chickens fed on the diet supplemented with evening primrose seeds expellers as 20% substitute of energy and protein show lower concentrations of Pb in the liver and kidneys after loading them *per os* with a single dose of lead acetate.

3. The activity of delta-amino-levulin dehydratase in the erythrocytes of chickens did not differ between the control and experimental group which may indicate the possibility of more intensive excreting or binding of Pb by phytochelates contained in the evening primrose seeds expellers.

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STRESZCZENIE

Badania przeprowadzono na brojlerach linii Astra B-2 żywionych paszą standardową – grupa kontrolna i paszą wzbogaconą do 20% wytłoczynami z nasion wiesiołka – grupa doświadczalna. Kurczęta obciążono jednorazową dawką octanu ołowiu (50 mg/kg masy ciała) podanego *per os*. Wątroby i nerki ptaków do oznaczeń stężeń ołowiu pobrano przed obciążeniem $Pb(CH_3COO)_2$, a następnie po: 1, 4 i 7 dobie po wywołanym zatruciu. Raz na dobę zbierano kał do analizy na zawartość Pb oraz pobierano od ptaków krew w celu oznaczenia aktywności dehydratazy delta-amino-lewulinianowej.

Maksymalne stężenie Pb w wątrobach i nerkach kur obu badanych grup wystąpiło po 1 dobie (6,28 µg/g wątroby – grupa kontrolna, 4,23 µg/g wątroby – grupa doświadczalna oraz 4,07 µg/g nerki – grupa kontrolna i 2,67 µg/g nerki – grupa doświadczalna). Stopniowo ilość ołowiu w wątrobach i nerkach ptaków malała i siódmego dnia wynosiła: 0,91 µg/g wątroby – grupa kontrolna, 0,67 µg/g wątroby – grupa doświadczalna, 2,04 µg/g nerki – grupa kontrolna i 1,37 µg/g nerki – grupa doświadczalna.

Kury z grupy doświadczalnej szybciej wydalały z przewodu pokarmowego jony Pb i w 7 dobie stężenie Pb w kale wynosiło 5,1 µg/g kału dla grupy kontrolnej i 3,6 µg/g dla grupy doświadczalnej.

Nie stwierdzono różnic w stopniu inhibicji aktywności dehydratazy delta-amino-lewulinianowej w erytrocytach kur obu grup ptaków poddanych obciążeniu octanem ołowiu.