

CLINICAL, HEMATOLOGICAL AND BIOCHEMICAL TESTS OF MALLARDS [ANAS PLATYRHYNCHOS, (L.)] FOLLOWING AN EXPERIMENTALLY INDUCED INTOXICATION WITH LEAD AMMUNITION

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ABSTRACT

Results from clinical, hematological and biochemical tests following an experimentally induced lead intoxication of mallards are presented. The clinical signs and the loss of body weight are proportional to the toxic exposition. Lower levels of red blood cells, hemoglobin and erythrocyte indices are registered. The results from the biochemical tests show elevated levels of liver transaminases, hypoproteinaemia, hypoalbuminaemia, and, also, significantly lower serum calcium levels.

Key words: mallards, intoxication, lead ammunition.

Introduction

The use of lead for manufacturing hunting ammunition dates back centuries and for thousands of years lead has been used in fishing. Nearly 150 species, representatives of the wildlife, that have been affected by lead intoxication due to ingestion of pellets, bullets or fragments of lead ammunition, are registered in scientific literature. Only in the late 19th century, hypotheses, evolving to indisputable facts, that proved that lead ammunition and fishing tackle are a major source of lead exposure expressed in toxicity with cumulative effect in game and fish, were formed. Despite the legal restrictions, significant amounts of lead ammunitions continue to be deposited in water and upland habitats thus entering the food chain up to the end consumer.

Lead is one of the most toxic metals and its negative effects range from mild biochemical and physiological disorders to serious pathological processes in which major organs and systems may be affected, with following functional and behavioral changes. The probability for a bird to be poisoned is determined by several factors, such as: time of retaining the lead elements, frequency of exposure, nutritional conditions, stress etc. There are documented cases of lethal poisoning as a result of only one pellet (Sanderson, G. C., and Bellrose, F. C. 1986). The gizzard of the bird, which contains swallowed sand from the river sediment (the so called gastrulites), is an ideal environment for the solubilization of lead. Furthermore, the gizzard combines acidic environment with strong contractibility of its cuticle, which as a result creates conditions for the abrasive action to lead, leading to its absorption for about 42 days (Pain et al., 2009). According to some scientists, lead fragments are readily consumed by the animals because of the salty-like taste of their oxidized surfaces, particularly when salt - deficient mammals and birds are concerned (Lewis et al. 2001). Most toxicologists determine levels of 16 mg/kg. BW lead from pellets as the cumulative lethal dose for birds (Dilov P., et al. 2005). Lead concentrations are the highest after direct absorption into the bloodstream, then in the kidneys and liver for days or months, and if the process becomes chronic the lead is deposited into the bones. If the intoxicated with lead birds are consumed by predators or saprophytes, the latter in turn absorb certain amounts of lead, which may result in their intoxication and death.

In terms of lead intoxication related to the use of lead hunting and fishing gear, in our country, there is no data of any conducted scientific studies with the exception of some popular scientific articles.

That is why we decided to investigate some of the toxic effects in waterfowl and in connection with their exposure to lead ammunition.

Material and Methods

16 clinically healthy mallards (*Anas platyrhynchos*) in the age range of 9 to 12 months and body weight range from 1050 to 1250 g were included in the experiment. The birds were divided by fours into 3 experimental and one control groups. They were kept separately, in groups, in closed aviaries with provided access to water for their physiological needs. Their feeding was organized with granulated feed mix for ducks provided twice a day. After a 7-day period of adaptation, the mallards were treated orally with lead pellets №3 with average weight of 0.26746 g as follows: 1st group – 3 lead pellets, 2nd group – 2 lead pellets, 3rd group – 1 lead pellet, and the 4th control group. The study was conducted over a period of 60 days after the treatment.

Bodyweight was recorded before treatment and then over seven-day intervals, respectively on the 7th, 14th, 21st, 28th, 35th and 42nd day.

Blood samples were obtained by venipuncture from v. subcutanea ulnaris before treatment, on the 7th, 14th, 21st, 28th, 35th and 42nd day. The obtaining of blood samples was performed using a closed system including needles MN-SV21Q (butterfly type) and 3ml tubes with Li- Heparin for hematology analysis and 5 ml tubes with Gel + Clot Act for biochemical analysis.

The hematology tests included: erythrocyte count (RBC), hemoglobin (Hgb), hematocrit (PCV), mean corpuscular volume (MCV), mean hemoglobin content in one erythrocyte (MCH), and the mean concentration of hemoglobin in erythrocytes (MCHC). The tests were performed with veterinary hematology analyzer Haemascreen 18.

The biochemical indicators included: serum levels of creatinine (Create), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), albumin (Albumin) and calcium (Ca2 +). For their determination biochemical analyzer Screen Master LIHD 113 was used.

The analysis and statistical processing of the data was performed by a computer program SPSS 19.0. The data are expressed as mean plus standard error.

In the current study the assessment was made with guaranteed probability of 0.95 (significance level = 0.05), whereas $p < 0.05$ was considered the lowest level of statistical reliability.

Results and Discussion

During the first two days (24–48 h.) after the treatment, there were no visible changes in the general condition and behavior of the mallards.

On the seventh day after the oral administration of the lead pellets the experimental birds from group I exhibit the first symptoms, such as green-colored diarrhea accompanied by increased thirst (polydipsia), and a decrease in body weight (Table 1).

During the time interval between the 7th–14th day in addition to the continuing greenish diarrhea, there is also a distressed locomotor activity, the eyes are narrowed (photophobia), and the mallards demonstrates vocal changes, resulting in deaf hissing sounds. There is a new regression in terms of body weight (Table 1).

A similar clinical finding is observed in the next five days (from the 14th to the 19th day after treatment), with deepening signs of polydipsia, accompanied by manifestations of adynamia and prostration.

During the last days before death, refractory cachexia, photophobia, vocal changes, lack of appetite and completely distressed locomotor activity with paralysis of limbs, and reliable weight loss (Roscoe et al., 1979), were registered (Table 1).

The mortality rate in the mallards from group I and groups II was 100 % as a result of a severe anemic syndrome.

In group III the mortality rate was 25 %, where cachexia, lethargy, greenish diarrhea, adynamia and prostration, serofibrinous conjunctivitis as well as reliable weight loss on the 35th - 42nd days after treatment, were observed (Table 1).

The loss of body weight, as well as the severity of clinical symptoms, was proportional to the dose of the applied lead. The diversity and nature of the manifested symptoms observed within each group of birds are due to the different individual sensitivity to the administered toxic dose.

Table 1: Change in the body weight for the different groups of the tested mallards (*Anas platyrhynchos*), reported before, on the 7th, 14th, 21st, 28th, 35th and 42nd day after oral administration of lead pellets.

Day	group I n = 4 x ± SE	group II n = 4 x ± SE	group III n = 4 x ± SE	Control group n = 4 x ± SE
Before	1185.75 ± 29.856	1215.00 ± 32.787	1122.50 ± 17.970	1088.75 ± 21.348
7 th	1110.00 ± 29.155	1130.00 ± 46.726	1088.75 ± 35.141	1122.25 ± 19.063
14 th	927.50 ± 46.615	1070.00 ± 39.158	1033.25 ± 58.628	1164.50 ± 15.793
21 st	822.50 ± 53.910**	970.00 ± 61.509*	933.75 ± 78.352	1192.00 ± 12.403
28 th	-	802.50 ± 80.661*	947.50 ± 124.908	1209.25 ± 12.665
35 th	-	725.00 ± 74.442*	945.75 ± 142.389*	1231.25 ± 11.404
42 nd	-	-	988.00 ± 156.466*	1251.50 ± 10.428

*p < 0.05; **p < 0.01; ***p < 0.001

Changes in the hematological parameters in mallards [*Anas platyrhynchos*, (L.)] following an experimentally induced lead intoxication.

There is a clear eritropeniya in the experimentally treated birds from group I on the 14th and 21st day, compared to the initial levels (Table 2).

In the birds from group II there is no registered statistical reliability, however, there is a tendentious decrease in the number of red blood cells (Table 2).

In the birds from group III a regressive trend in the total number of red blood cells up to the 28th day, followed by a gradual slow normalization correlating with cessation of clinical signs, was observed (Table 2).

In the control group, the fourth group, the number of red blood cells is maintained within the reference levels (Table 2).

A decrease in the concentrations of hemoglobin (Hgb) and hematocrit (PCV) indicators was observed (Table 3).

Significant regression in terms of hemoglobin is present mostly in the experimentally treated mallards from group I and group II, which correlates with the decreased levels of red blood cells (Table 2). This decrease is associated with the ability of the lead to inhibit the sulfhydryl enzymes involved in the cellular metabolism, leading to the deposition of hemoglobin precursors (Dilov P. et al. 2005; Nikov, 1977; Anderson, et al., 1985; Fisher et al., 2006).

Table 2: Effect of the experimental lead poisoning on the average numbers of erythrocytes, hemoglobin and hematocrit level in mallards [*Anas platyrhynchos*, (L.)].

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
I	RBC	3.3450	2.0450	1.2400	1.2550			
	(10 ¹² /L)	± 0.08180	± 0.07100	± 0.15226*	± 0.15300*	–	–	–
	Hgb	197.00	125.50	72.75	59.50			
	(g/L)	± 2.082	± 4.406	± 10.274**	± 4.330*	–	–	–
	PCV	52.600	34.600	21.050	21.300			
II	(%)	± 3.0586	± 1.7903*	± 3.0937	± 2.0785	–	–	–
	RBC	3.1125	2.7075	2.4175	2.1125	1.6475	1.5225	
	(10 ¹² /L)	± 0.59263	± 0.09031	± 0.11191	± 0.10451	± 0.09595	± 0.03425	–
	Hgb	205.75	160.75	133.00	107.50	79.25	64.25	
	(g/L)	± 14.203	± 1.931*	± 3.136	± 7.511	± 9.543	± 2.562	–
III	PCV	55.600	48.925	44.225	34.600	26.250	22.575	
	(%)	± 1.3620	± 1.7433	± 1.3028	± 1.5039	± 0.9474	± 1.3143	–
	RBC	2.7300	2.2275	2.1600	2.0200	2.2950	2.2650	2.5225
	(10 ¹² /L)	± 0.18471	± 0.40485	± 0.25103	± 0.30469	± 0.44558	± 0.43202	± 0.51367
	Hgb	193.50	127.00 ±	125.25	118.75	133.00	146.25	166.25
Contr.	(g/L)	± 12.926	24.782	± 19.163	± 18.495	± 31.115	± 30.090	± 40.463*
	PCV	45.450	36.800	35.125	31.725	39.250	34.350	35.925
	(%)	± 3.7369	± 7.0075	± 4.6245	± 4.6015	± 10.3159	± 6.1637	± 6.6649
	RBC	2.7425	3.2625	2.7300	2.5950	2.9450	2.9450	2.8525
	(10 ¹² /L)	± 0.20882	± 0.34038	± 0.25096	± 0.03329	± 0.13629	± 0.05867	± 0.08693
	Hgb	182.50	187.50	169.75	195.75	186.74	188.50	177.25
	(g/L)	± 7.599	± 6.564	± 13.269	± 6.074	± 5.642	± 5.694	± 1.109
	PCV	46.625	56.250	40.825	40.325	44.575	50.250	48.300
	(%)	± 4.2927	± 6.0207	± 3.2451	± 1.1996	± 1.3325	± 2.0060	± 0.8175

*p < 0.05; **p < 0.01; ***p < 0.001

Regarding the erythrocyte indices (MCV, MCH and MCHC), there is a characteristic decrease compared to the initial levels in group I and group II, treated with a higher lead concentration (Table 3). The decrease of the values of the erythrocyte indices is an indication of the presence of microcytic anemia (Angelov 1999; Del Bono, 1973).

Table 3: Effect of the experimental lead poisoning on the erythrocyte indices (MCV, MCH, MCHC) in mallards [*Anas platyrhynchos*, (L.)].

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
I	MCV	169.00	170.75	140.50	162.50			
	(fL)	± 1.472	± 1.887	± 4.907***	± 1.732**	–	–	–
	MCH	61.000	53.350	52.700	45.100			
	(pg)	± 1.1083	± 4.3007***	± 3.3919	± 0.6351	–	–	–
	MCHC	369.25	312.50	328.00	297.00			
II	(g/L)	± 7.521	± 27.600***	± 20.314	± 9.238	–	–	–
	MCV	162.15	172.50	160.25	158.75	155.25	145.50	
	(fL)	± 6.359	± 2.754	± 3.425	± 3.591	± 2.689	± 8.995	–
	MCH	68.750	56.050	58.000	52.075	50.525	46.025	
	(pg)	± 3.1071	± 1.1850	± 1.8453	± 3.0341	± 2.0483	± 3.8947	–
	MCHC	431.75	334.25	366.25	330.00	324.25	319.75	
	(g/L)	± 29.219	± 13.762	± 7.432*	± 20.765	± 15.456	± 35.056	–

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
III	MCV (fL)	166.43 ± 4.084	165.75 ± 3.568	159.00 ± 5.788	156.00 ± 6.843	153.50 ± 5.605	157.25 ± 1.377*	159.00 ± 2.121
	MCH (pg)	72.300 ± 7.6183	57.750 ± 4.3448*	55.300 ± 4.3507*	55.675 ± 2.0786**	49.675 ± 3.2268**	62.950 ± 6.0506	66.250 ± 7.2407
	MCHC (g/L)	439.00 ± 55.562	348.50 ± 23.659*	347.00 ± 18.855**	372.75 ± 6.613***	408.50 ± 15.398**	406.75 ± 25.539*	393.50 ± 25.168
	MCV (fL)	167.43 ± 3.735	170.25 ± 1.702	154.25 ± 4.785	154.75 ± 3.351	162.25 ± 2.175	167.25 ± 1.548	161.00 ± 1.683
	MCH (pg)	64.175 ± 5.1611	58.550 ± 1.5414	63.400 ± 1.3466	75.025 ± 2.6129	65.675 ± 1.3047	61.550 ± 1.2978	57.900 ± 1.6299
Contr.	MCHC (g/L)	342.50 ± 6.171	360.00 ± 5.492	409.75 ± 5.893	478.00 ± 28.726	406.25 ± 7.642	362.00 ± 9.381	333.00 ± 9.764

*p < 0.05; **p < 0.01; ***p < 0.001

Changes in the biochemical parameters in mallards [*Anas platyrhynchos*, (L.)] following an experimentally induced lead intoxication.

When examining the transaminases ASAT and ALAT (Table 4) elevated levels of statistical reliability were observed, which is an indication of degenerative changes in the liver (Angelov, 1999; Mircheva, 2005; Mateo et al. 2003).

Alkaline phosphatase (AP) is an indicator of the osteoblastic activity and lead exposure is associated with alteration and mineralization of the bones in birds (Gangoso et al., 2009, Martinez-Haro et al. 2011).

In the mallards from group I, the increased concentration of lead in the blood is accompanied by reduced activity levels of the AP, where values remained within the reference levels (Mateo et al. 2003 b). In the experimental birds from group III (treated with lower doses) there is a statistically significant increase in the levels of AP which suggests a chronic process and deposition of lead in the bones.

Table 4: Effect of the experimental lead poisoning on the liver transaminases level in mallards [*Anas platyrhynchos*, (L.)].

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
I	ASAT (UI/l)	27.000 ± 9.1042	81.250 ± 13.7721	115.675 ± 7.4366	125.450 ± 5.2250	—	—	—
	ALAT (UI/l)	36.825 ± 5.5552	59.450 ± 8.9245	77.575 ± 10.8992	78.600 ± 9.0067*	—	—	—
	AP (UI/l)	201.775 ± 29.6609	161.000 ± 32.2417	120.500 ± 22.9658	170.950 ± 52.3044*	—	—	—
	ASAT (UI/l)	32.600 ± 0.0000	60.625 ± 14.4899*	104.975 ± 7.4964**	117.225 ± 7.5260	96.950 ± 5.0419*	95.800 ± 6.1124*	—
	ALAT (UI/l)	34.225 ± 8.0257	47.525 ± 1.2776*	53.500 ± 3.0367	54.125 ± 7.2721	63.475 ± 5.2706	66.950 ± 5.5917	—
II	AP (UI/l)	209.250 ± 15.7606	192.750 ± 20.7988	133.525 ± 30.4952	163.900 ± 19.7593	184.775 ± 12.2918	174.250 ± 8.9040	—
	ASAT (UI/l)	18.100 ± 2.7249	68.900 ± 2.8499	101.700 ± 9.1310*	113.900 ± 11.3428**	99.875 ± 8.9141*	72.300 ± 18.2311	78.775 ± 16.0383
III	ALAT	49.650	46.800	72.200	70.575	62.100	54.750	60.400

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
Contr.	(UI/l)	± 1.0524	± 3.5221	± 5.7881*	± 11.2970 **	± 13.5584 ***	± 12.4656*	± 10.3734*
	AP	182.100	180.125	270.500	217.625	186.525	205.100	218.725
	(UI/l)	± 3.2104	± 9.7643*	± 14.1667*	± 50.9784*	± 35.6294*	± 29.7565*	± 33.9074*
	ASAT	22.325	43.750	39.875	23.925	33.825	40.475	37.550
	(UI/l)	± 0.9040	± 4.5688	± 4.5428	± 4.2074	± 3.568	± 1.7542	± 0.9430
	ALAT	51.250	47.950	43.300	46.925	50.100	47.100	49.875
	(UI/l)	± 1.3525	± 1.9504	± 1.0320	± 1.2822	± 1.5061	± 0.8436	± 1.4250
	AP	186.050	196.425	189.650	194.925	191.775	194.550	184.225
	(UI/l)	± 3.7078	± 7.3846	± 1.7576	± 2.1093	± 1.8621	± 2.4510	± 1.9491

*p < 0.05; **p < 0.01; ***p < 0.001

In the experimental birds from group I the creatinine levels are maintained within the norm, furthermore, there is even a visible decrease which is associated with the presence of an anemic syndrome (Angelov, 1999). Whereas, in group II and especially experimental group III there is an increase in the creatinine level which could be an indication of degenerative changes in the kidneys (Angelov, 1999; Mateo et al., 2003a). But, overall, the creatinine levels in this study were within the normal range (Table 5).

The examination of the serum proteins showed a slight but sustained decrease in the albumin levels, characteristic especially for the first and second experimental groups, in which the administered toxic dose was higher (Del Bono, 1973). This implies impaired resorption on behalf of the digestive system along with signs of anemia (Angelov, 1999; Mircheva, 2005).

There was a significant decrease of the levels of serum calcium compared to its initial values. This could be due to a series of biochemical regulatory mechanisms at the cellular level and the suppressive effect of the lead in relation to the calcium (Gangoso et al., 2009, Martinez-Haro et al. 2011).

Table 5: Effect of the experimental lead poisoning on the average numbers of creatinine, albumin and calcium levels in mallards [*Anas platyrhynchos*, (L.)].

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
I	Creat.	48.600	41.975	25.925	25.900			
	(µmol/l)	± 15.6907	± 5.7686	± 4.4252	± 4.4336	—	—	—
	Albumin	19.375	16.100	14.125	16.525			
	(g/l)	± 1.1302	± 1.7345*	± 1.5140	± 2.8482	—	—	—
	Calcium	2.000	1.900	1.475	1.350			
II	(mmol/l)	± 0.1472	± 0.0816	± 0.1109	± 0.0645**	—	—	—
	Creat.	57.525	39.825	34.875	58.350 ±	48.100 ±	34.725	
	(µmol/l)	± 14.3325	± 7.4170	± 7.8711	22.1528	15.9399	± 8.2060	—
	Albumin	23.575	19.250	20.225	18.975	15.350	13.500	
	(g/l)	± 2.9004	± 0.8549	± 1.2645	± 1.5526	± 1.4540	± 0.5431	—
III	Calcium	2.400	1.850	2.100	1.400	1.375	1.175	
	(mmol/l)	± 0.2160	± 0.1658	± 0.1581	± 0.0408	± 0.2926	± 0.1887	—
	Creat.	37.525	34.425	29.775	35.850	26.100	27.675	51.050
	(µmol/l)	± 2.1124	± 3.6684	± 1.7143	± 4.0891	± 4.0301	± 4.2820	± 12.1442
	Albumin	19.650	16.575	19.075	20.250	18.825	17.825	18.550
	(g/l)	± 1.0300	± 0.8499	± 1.4221	± 2.1562	± 2.4356	± 2.2051	± 2.2897

Group	Index	Before n = 4 x ± SE	7 th day n = 4 x ± SE	14 th day n = 4 x ± SE	21 st day n = 4 x ± SE	28 th day n = 4 x ± SE	35 th day n = 4 x ± SE	42 nd day n = 4 x ± SE
Contr.	Calcium (mmol/l)	1.850 ± 0.1323	1.850 ± 0.1848	2.000 ± 0.2345	1.198 ± 0.0931	1.765 ± 0.3404	1.650 ± 0.2255	1.700 ± 0.2415
	Creat. (μmol/l)	37.775 ± 1.8085	38.800 ± 1.7335	34.650 ± 1.5174	31.125 ± 1.5151	33.850 ± 1.8012	41.000 ± 1.6477	48.225 ± 1.2526
	Albumin (g/l)	20.875 ± 0.9936	21.375 ± 0.3728	21.700 ± 0.6014	21.725 ± 0.6019	21.925 ± 0.5573	20.400 ± 0.4708	20.800 ± 0.1871
	Calcium (mmol/l)	2.000 ± 0.1080	1.900 ± 0.1291	2.200 ± 0.1080	1.800 ± 0.1080	2.250 ± 0.1555	2.100 ± 0.1080	2.075 ± 0.1250

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Conclusions

The clinical signs and loss of body weight, proportionally correlates to the size of the administered toxic dose.

The results from the hematological and biochemical analyses show the presence of macrocytic (normochromic) anemia and possible liver and kidney degenerative changes as well as deposition of lead in the bones.

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