Baltic Coastal Zone No. 13	
(187-197) 2009	Institute of Biology and Environmental Protection Pomeranian Academy Słupsk

OXIDATIVE STRESS AND PROTEIN OXIDATION AFFECTED BY TOXIC METALS IN FERAL PIGEONS (*COLUMBA LIVIA*) FROM NORTHERN POLAND

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Abstract

The aim of this study was to compare ecophysiological basis for developing feral pigeons (*Columba livia f. urbana*) in various environments of Northern Poland. We examined heavy metals contents, lipid and protein peroxidation, antioxidant enzymes activity in individuals growing and feeding in the different polluted regions. Pigeons from urban area possessed high maintenance of cadmium in the blood, but low lead in comparison to birds from rural area. Our results suggest that increased level of heavy metals (Pb and Cd) in the blood of pigeons from different regions of Northern Poland tended to affect negatively initiate lipid peroxidation and increased oxidative modified protein content. Our results suggest that increased level of oxidative stress in birds is dependent upon environmental pollution. Statistical analysis (ANOVA and GLM) has shown that colony localization (urban or rural areas) modified antioxidative defense system, level of lipid and protein peroxidation, and blood total antioxidant activity.

Key words: feral pigeon, C*olumba livia*, lipid peroxidation, oxidative stress, antioxidative enzymes, environment, toxic heavy metals

INTRODUCTION

Ecotoxicological studies of pollutants in ecosystems affect the organisms and describe the population and ecosystem structure. Living birds have been shown to be particularly useful bioindicators because they are visible, sensitive to toxicants and

they rank relatively high in the food chain and are important structural components of the ecosystem (Nam et al. 2004a). They therefore give early warnings of environmental stress and are of general interest to the public.

A feral pigeon is represented in most cities on all continents of the Earth. Its colonies are often saved in the center of cities. No wonder that large accumulations of these birds often appear in the neighborhood of intensive car traffic. Research by Hetmański (2007) shows that it is the settled bird, not moving in other places, and adults are largely tied to certain breeding colony. His observations have shown that 30% of young birds keep paternal colony, that always moves on territory of the same city. In opposition adults have the high relationship with a birthplace. Such lines of pigeons allow to be a biomarker for pollutant mediated oxidative damage of areas, such as domestic sparrow *Passer domesticus* (Swaileh and Sansur 2006), for example. Estimation of bioaccumulation level of substances in the body of pigeons allows to represent the scale of contamination of cities nearby the residence and feed area.

The use of pigeons in researches on the contamination level of toxic substances in large and small industrial towns is an open question. Many reviews reported that pigeons in large agglomerations have the higher level of cadmium and lead in tissues compared with birds in small towns or rural area (Nam et al. 2004b, Nam and Lee 2005, 2006a, b). It deals with traffic density as reported by Garcia et al. (1988) and Schilderman et al. (1997). Accumulation of heavy metals takes place unevenly in tissues of birds. The highest lead concentration was found in the bone followed by kidney, liver and lung of pigeons (Johnston and Janiga 1995, Nam and Lee 2005). That is not subject to doubting that there are substantial differences between the levels of heavy metals between eggs, young and old individuals. Nam et al. (2004a) showed that there was multiplied maintenance of lead from eggs through nestling and the greatest in adult pigeons. It has been noted the difference of bioaccumulation of toxic substances between males and females. This phenomenon is shown also in other types of birds, e.g. pelican females (Donaldson and Braune 1999).

First research reporting the influence of heavy metals on physiological processes at pigeons belongs to Hutton (1980). This author first connected the high level of lead at pigeons from the center of London with changes of their mitochondrial structure, function and depression of delta-aminolevulinate dehydratase activity in blood, liver and kidney. Other authors showed direct influence of lead on oxidative DNA damage (Schilderman et al. 1997).

The aim of this study was to compare ecophysiological basis for developing feral pigeons (*Columba livia f. urbana*) in various Northern Poland environments. We examined heavy metals contents, antioxidant enzymes activity in individuals growing and feeding in the different polluted regions. Thus we investigated the content of proteins carbonyl concentration, lipid peroxidation and superoxide dismutase, catalase, ceruloplasmine, glutathione peroxidase and glutathione reductase activities in blood of birds from these environments.

MATERIALS AND METHODS

Study area

Researches were conducted in two regions: first was the Słupsk city (urban area) and second was the Szpęgawa village (rural area) near the Tczew city (N Poland). Słupsk is a city with population about 100 000 habitants. Pigeon population has about 1 200 individuals (data of 2006 year). The colonies of the Słupsk pigeons are located in Old Market city center. The herd of such birds counted to 500 individuals. The second group was from Szpęgawa and counted 60 adults. The farmer territory with birds' herds was placed near a street with intensive car traffic of the Skarszewy–Tczew route. Few kilometers from this farm it was organized intensive road linked with building of A1 Poland motorway. In this connection in the last two years near the farmer economy location the high level of heavy motor transport was observed.
The experiments were conducted with the Guidelines of the European Union Council and the current laws in Poland, according to the Ethical Commission (permission number: 05/2005).

Metal analysis

Adult feral pigeons were collected during 2006 to examine lead and cadmium concentrations in venous blood samples. The levels of these elements in serum were measured by Atomic Absorption Spectrometry (AAS) with the Perkin-Elmer Instrument (type RW 683/3PYC). Calibration curves were constructed using Merck standards. The detection of elements is around the mg/L level.

Blood samples preparation and lipid peroxide level

Blood samples were centrifuged for 10 min at 18 700 g and plasma was pipetted off. Pipetted 1:20 hemolysate of plasma was used for determination of the malondialdehyde level, oxidative modification of plasma proteins, total antioxidant activity of blood and catalase activity.
Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reactive substances (TBARS) concentration (micromole per liter of blood), it was done as described by Uchiyama and Mihara (1978). Carbonyl groups formed from oxidation with 2.4-dinitrophenyl hydrazine (DNPH) were estimated using the methods by Levine et al. (1990) with modifications (Dubinina et al. 2000). Estimation of derivatives of 2.4-dinitrophenyl hydrazones in blood may serve as a pattern of oxidative modification of proteins during oxidative stress in cells. The levels of total antioxidant activity in the blood were estimated following the method with Fe^{2+} and ascorbate-dependent Tween-80 oxidation using method by Halaktionova et al. (1998). Superoxide dismutase (SOD; E.C. 1.15.1.1) activity was measured by the quercetine method after suitable dilution following the method by Kostyuk et al.

(1990). The catalase activity was evaluated using the hydrogen peroxide breakdown method (Korolyuk et al. 1988). Glutathione reductase (GR; E.C. 1.6.4.2) activity was determined by reduced NADPH and oxidized glutathione (GSSG) substrates in the 1:20 hemolysate measured according to the method described by Glatzle et al. (1974). Glutathione peroxidase (EC 1.11.1.9) activity was measured following the method of Moin (1986).

Statistical analysis

The results were expressed as mean ±SD. Significant differences among the means were measured using a multiple range test at min. $p<0.05$. The variations in each biomarker and metal concentrations were tested by one-way analysis of variance (ANOVA), considering sites as variables (Zar 1996). When ANOVA revealed significant differences, post-hoc multiple comparisons between sites and between biochemical parameters were made using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine which values differed significantly. ANOVA and general linearized modeling (GLM) were used to study the estimation of cadmium and lead influence on pro- and antioxidant balance in blood of pigeons. Tests were conducted with SAS 9.1 program for Windows.

RESULTS

Cadmium and lead levels in blood of pigeons are presented in figure 1. Pigeons from urban area had high level of cadmium (by 53.4%, $P<0.05$), but low lead level in blood in comparison to that of birds from the rural area (Szpęgawa). Vice versa, birds from rural area were characterized by a high lead level (185.2%, $P<0.001$) in blood in comparison to that of pigeons from the urban area.
Our results suggest that increased level of heavy metals (Pb and Cd) in pigeon's blood from different regions of Northern Poland tended to negatively affect initiate

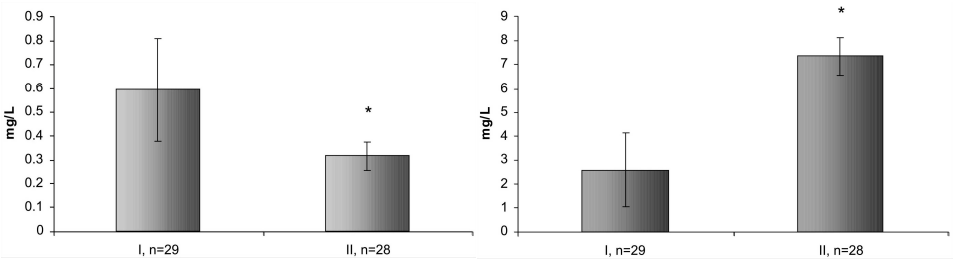


Fig. 1. Cadmium (A) and lead (B) levels in the plasma of feral pigeons (*Columba livia*) from different regions of Northern Poland (means ±SEM)

* – Statistical difference between samples taken in Słupsk (I) and Szpęgawa (II) by paired t-test ($P<0.001$)

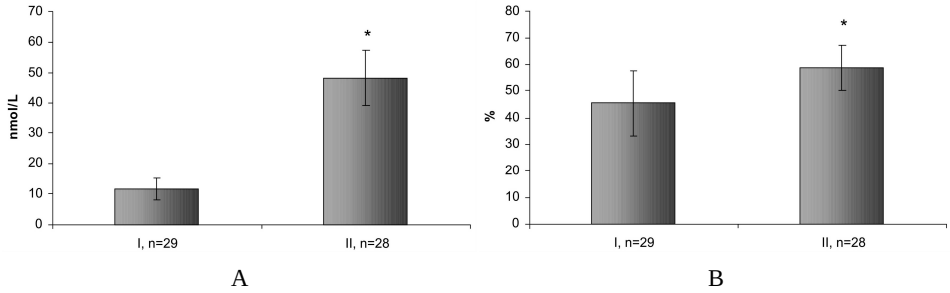


Fig. 2. TBARS concentrations (A) and total antioxidant activity (B) in the blood of feral pigeons (*Columba livia*) from different regions of Northern Poland (means ±SEM)
* – for legend, see Fig. 1

lipid peroxidation, estimated by thiobarbituric acid reacting substances (TBARS) concentration, the last product of lipid peroxidation, expressed as malondialdehyde (MDA) concentration. The level of MDA formation in blood of pigeons is shown in figure 2A. MDA level was significantly elevated in blood of pigeons from rural area (Szpęgawa) compared to that of Słupsk. The percentage increase in lipid peroxidation compared to pigeons from Słupsk was about 4 times higher ($P < 0.001$).
A rate of protein oxidative destruction may be estimated from the reaction of the resultant carbonyl groups of amino acids (ketones and aldehydes) reaction with 2.4-dinitrophenyl hydrazine to produce 2.4-dinitrophenylhydrazones. The initiating effect of nonenzymatic components (Fe^{3+}-ascorbic acid, Fe^{2+}-O_2, Fe^{2+}-H_2O_2) was in the metal-catalyzing oxidation of proteins. Figure 3 shows the levels of 2.4-dinitrophenylhydrazone (aldehyde and ketone derivatives) in blood of pigeons from different Northern Poland regions.

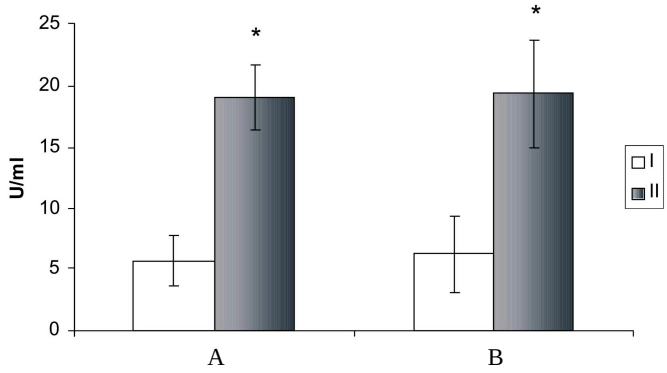
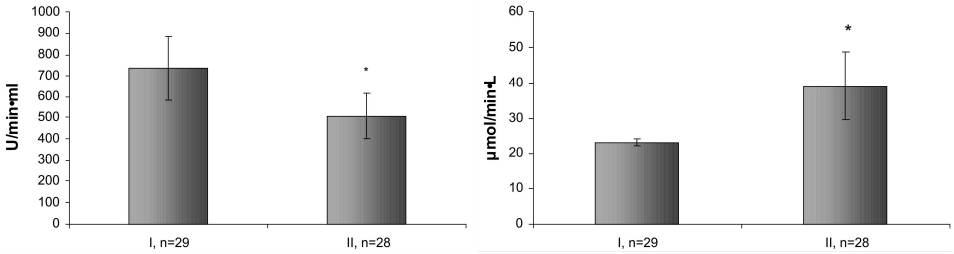


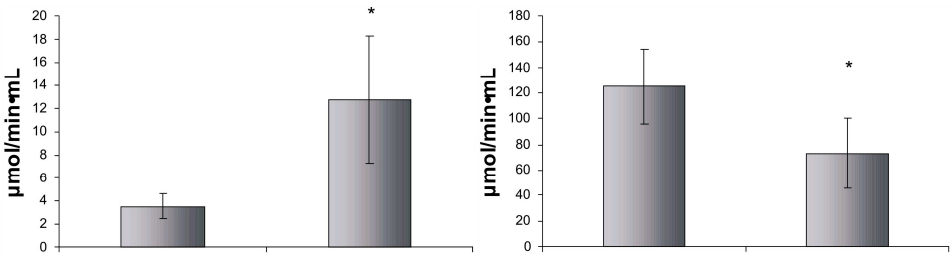
Fig. 3. Level of oxidative modified proteins (contents of aldehyde and ketone derivatives) in the blood of pigeons (*Columba livia*) from different regions of Northern Poland. Carbonyl groups were derivatived with 2.4-dinitrophenylhydrazine and determined spectrophotometrically from the difference in absorbance at 370 nm (A) and 420 nm (B), (means ±SE calculated from two independent experiments)
* – for legend, see Fig. 1



A B

Fig. 4. Superoxide dismutase (A) and catalase (B) activities in the blood of feral pigeons (*Columba livia*) from different regions of Northern Poland (means ±SEM)

* – for legend, see Fig. 1



A B

Fig. 5. Glutathione peroxidase (A) and glutathione reductase (B) in the blood of feral pigeons (*Columba livia*) from different regions of Northern Poland (means ±SEM)

* – for legend, see Fig. 1

A rate of protein oxidative destruction increased significantly in the blood of pigeons from the village compared with that of pigeons from Słupsk. The percentage increase in 2.4-dinitrophenylhydrazones levels was about 300% higher ($P<0.001$). Our results suggests an increased level of oxidative stress in birds as a result of toxic action of the environment.

The results of enzyme activities of pigeons group from urban and rural areas were shown in figures 4, 5. The data from this study indicate that pigeons from rural areas may be exposed to oxidative stress in blood. Comparison between Słupsk and Szpęgawa group of pigeons revealed differences in enzyme activities. SOD activity was significantly decreased in all samples of Szpęgawa group ($P<0.001$, 31% of Słupsk group). A fall in the activity of SOD may be due to inactivation by interaction with oxygen radicals. Glutathione peroxidase and catalase activities increased (about 4 and 1.5 times higher in comparison to Słupsk pigeons group, $P<0.001$, respectively) in the group of pigeons from rural areas.

Glutathione reductase activity decreased ($P<0.001$) about 150% in pigeons from rural area (Szpęgawa) with respect to the Słupsk group (Fig. 5B). Regarding the total antioxidant activity (Fig. 2B), its level was significantly increased ($P<0.05$) – about 20% in the blood of Szpęgawa group with respect to that of Słupsk.

ANOVA and general linearized modeling (GLM) were used for answering the question: what parameters of environment or type of environment pollution have impact on investigated parameters? First, the type of colony caused the greatest influence on differences of pro- and antioxidative balance in the blood of pigeons from different regions of Northern Poland. The total analysis for all data with statistical difference is summarized in the Table 1. The analysis is conducted for all individuals of birds included. These data showed, that colony localization (urban, rural areas) modified statistically the SOD and glutathione reductase activity, MDA concentration, neutral and alkaline 2.4-dinitrophenylhydrazones content. Neither lead nor cadmium influenced significantly these parameters.

Differences between determination in cadmium and lead levels in pigeon's colonies (from urban and rural areas) define the next step – how concentration of these metals can influence the level of oxidative stress? It has been shown the dependence of cadmium level (low concentration of this metal in blood was noted) and activity

Table 1

The influence of colony localization and lead and cadmium levels upon antioxidant status in feral pigeons from different regions of Northern Poland (ANOVA)

Source	Dependent variable						
	Catalase	MDA	Neutral DNPH	Alkaline DNPH	Glutathione peroxidase	SOD	Glutathione reductase
Pb	0.13	0.93	0.57	0.81	0.69	0.01	0.05
Cd	0.79	2.13	0.49	0.49	0.87	0.08	0.02
Colony	53.37[c]	49.49[c]	29.66[c]	12.81[b]	2.67	4.35[a]	8.71[b]

[a] – $P<0.05$, [b] – $P<0.01$, [c] – $P<0.001$

Table 2

GLM analysis of Cd and Pb influence upon antioxidant balance in the blood of pigeons from different Poland regions

Source	Dependent variable						
	Szpęgawa						
	Catalase	MDA	Neutral DNPH	Alkaline DNPH	Glutathione peroxidase	SOD	Glutathione reductase
Pb	5.01[a]	5.95[a]	0.11	5.79[a]	0.13	7.64[a]	0.85
Cd	0.04	1.18	2.95	0.275	0.88	1.05	0.33
Słupsk							
Pb	0.06	0.00	0.06	0.34	1.50	0.01	0.06
Cd	0.25	0.48	0.73	0.00	0.03	0.02	0.01

[a] – $P<0.05$

of superoxide dismutase, catalase, and neutral and alkaline 2.4-initrophenylhydrazones content in the blood of Szpęgawa pigeons (Tab. 2). Such dependence was not defined for birds from city colony. It is necessary to point that no interdependence between parameters of antioxidant defense system was stated. Simultaneously low (pigeons from Słupsk) and high (pigeons from Szpęgawa) levels of lead in the blood was stated.

Statistical analysis of our research allows to conclude the high tension of antioxidant defence system, caused by high TBARS and oxidatively modified proteins contents in the blood of birds from the village. But simultaneously, we show the increase of total antioxidant activity (Fig. 2B) in the blood of the same birds. Statistical GLM analysis showed, that it is conditioned by a high activity of catalase, but not glutathione peroxidase. It is necessary to mark that the activity of this last enzyme also increased considerably. Possibly, at this level of antioxidant defence the catalase enzymatic activity (from all showed parameters) in the greatest degree retains an adaptation of equilibrium between products and elimination of ROS in the blood of pigeons.

DISCUSSION

The main goal of our study was to compare metal concentrations, level of lipid and protein peroxidation, antioxidative enzyme activities in the blood of feral pigeons (*Columba livia*) from different regions of Northern Poland (urban polluted and rural area). We expected this species to be a useful indicator of point-source contamination in terrestrial ecosystems, suggesting that metal levels, lipid and protein peroxidation, antioxidative enzyme activities in the blood of adults can be used as indicators of local exposure.

There were very large differences between the two sites for lead, which was 3 times higher in rural area (Szpęgawa), than in polluted Słupsk places. These very high lead levels in blood of pigeons from the rural area (Szpęgawa) were the most surprising conclusion from our study. In fact, birds in an urban area are also in a direct closeness to cars in the city center. We took into consideration that rural pigeons are subjected to a higher pression of car traffic along the motorway A1 building. Our data are in agreement with the previous studies of Schilderman et al. (1997), Nam and Lee (2006) and Nam et al. (2004b). They demonstrated that high traffic exhaust tends to be one of the major lead sources (>50% contribution of Pb from gasoline), despite the use of lead-free gasoline, in the blood of urban pigeons from the Netherlands.

In our study the blood cadmium concentration in pigeons from Słupsk was about 2 times higher that in thouse from Szpęgawa. These results demonstrated that pigeons from urban (Słupsk) environment have been significantly contaminated.

Based on statistical GLM analysis, our results clearly showed that lead concentration led to oxidative stress, with pigeons from rural area Szpęgawa showing a 5-fold increase of MDA in blood with respect to pigeons from Słupsk. Some toxic effects of lead have been attributed to lead-induced oxidative stress. Results from our study

testify an enhanced MDA content and levels of oxidative modified proteins in pigeons from Szpęgawa with increased lead level. The increases in lipid and protein peroxidation were accompanied by alterations in their antioxidant defense systems (McGowan and Donaldson 1986), including decreased glutathione reductase and SOD activity and increased catalase and glutathione peroxidase activity (Fig. 1-5, Tab. 1-2).

Lead has been reported to cause oxidative stress by disrupting the intracellular prooxidant/antioxidant balance that exists in normal tissues (Ercal et al. 1996, Gurer and Ercal 2000). Participation of iron in Fenton reaction in vivo, leading to production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 (Free radicals… 1989) results in increased lipid peroxidation. Our previous studies suggest that exposure of rats on lead caused a reduction in glutathione peroxidase and reductase activities and lowered cellular defense against oxidative stress (Tkachenko et al. 2007). Increased activity of catalase in the present finding could be correlated with increased generation of hydrogen peroxide in lead exposed pigeons from Szpęgawa.

Our results indicate that degree of protein oxidative destruction increased significantly in the blood of pigeons from the rural area compared to that of pigeons from Słupsk. The percentage increase in 2.4-dinitrophenylhydrazones levels was about 300% higher ($P < 0.001$). The oxidative modifications of proteins play a crucial role in the pathological response of cells to increased oxidative stress.

CONCLUSIONS

Our results indicate the increased level of oxidative stress and modifying antioxidant enzymes activities in birds upon toxic action of environments from Northerm Poland.

Our results suggest that increased level of heavy metals (Pb and Cd) in the blood of pigeons from different regions of Northern Poland tended to affect negatively the initiative lipid peroxidation and increased oxidative modified protein content.

Pigeons from urban area (Słupsk city) possessed high maintenance of cadmium in the blood, but low lead in comparison to birds from rural area.

Our results suggest that increased level of oxidative stress in birds is dependent upon environmental pollution.

Statistical analysis (ANOVA and GLM) has shown that colony localization (urban or rural areas) modified antioxidative defense system, level of lipid and protein peroxidation and total blood antioxidant activity.

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STRES OKSYDACYJNY I OKSYDACYJNA MODYFIKACJA BIAŁEK POD WPŁYWEM OŁOWIU I KADMU U GOŁĘBI Z RÓŻNYCH ŚRODOWISK PÓŁNOCNEJ POLSKI

Streszczenie

Celem badań była analiza ekofizjologiczna postaw rozwoju dzikich gołębi (*Columba livia f. urbana*) w różnych środowiskach północnej Polski. Przebadaliśmy zawartość metali ciężkich, poziom procesów lipoperoksydacji, oksydacyjnej modyfikacji białek oraz aktywność enzymów antykoksydacyjnych u gołębi rozwijających się w środowiskach o różnym stopniu antropopresji. Krew gołębi pochodzących z miejskiego obszaru charakteryzowała się zwiększoną zawartością kadmu, a obszarów wiejskich – ołowiu. Wyniki naszych badań sugerują, że zwiększenie poziomu metali ciężkich ↑ (Pb i Cd) we krwi gołębi różnych regionów północnej Polski prowadzi do intensyfikacji procesów lipoperoksydacji, oksydacyjnej modyfikacji białek i zależy od stopnia zanieczyszczenia środowiska. Statystyczna analiza (ANOVA i GLM) wykazała, że lokalizacja kolonii (miejskie albo wiejskie obszary) wpływa na zmiany parametrów funkcjonowania systemu pro- i antyutleniania, modyfikuje mechanizmy obrony antyoksydacyjnej – całkowitą zdolność antyoksydacyjną.