CHOSEN BLOOD BIOCHEMICAL PARAMETERS IN FREE-LIVING WILD AND FARMED MINKS, FOXES AND RACCOON DOGS

Bożena Nowakowicz-Dębek, Andrzej Zoń[1], Andrzej Jakubczak[2], Wioletta Wnuk

Department of Animal and Environmental Hygiene,
Faculty of Biology and Animal Breeding, University of Life Sciences
Akademicka 13, 20-950 Lublin, Poland
[1]National Research Institute of Animal Production, 39-331 Chorzelów 771, Poland
[2]Department of Biological Basis of Animal Production,
Faculty of Biology and Animal Breeding, University of Life Sciences
Akademicka 13, 20-950 Lublin, Poland

Corresponding autor: E-mail: bozena.nowakowicz@up.lublin.pl; Tel. 0048, 81-445-69-98

Abstract. The objective of the study was comparison of chosen biochemical parameters in the blood of raccoon dogs, foxes and free-living wild and farmed minks. The research aimed at investigating the effect of different environments of the farmed and free-living animals on chosen blood parameters that could be indicators of potential health problems of animals. For collection of blood samples, wild animals were captured and housed under farm conditions. Biochemical analysis of blood plasma was performed using a MINDRAY BS-130 chemistry analyzer. There were found divergences in the analyzed blood parameters in the farmed and free-living animals. Statistically significant differences were determined estimating the ALT, AST and LDH activity levels in raccoon dogs and minks, while for foxes, statistical significance was noted between ALT, AST, BiLT and UREA concentration. The differences between the analyzed parameters in the farm-raised animals and in those living in the wild point out adaptive separateness of these animals. Information on the analyzed blood biochemical parameters of the free-living population is very facilitative allowing maintaining farm animals' welfare and obtaining high performance.

Keywords: biochemical parameter, farmed animal, fox, mink, raccoon dogs, free-living animal

Introduction. The scale of fur animal farming has stimulated the development of fur industry for years. In the early times, furbearing animals were trapped to obtain peltry but as they did not satisfy the producers' expectations /requirements, the efforts were undertaken to improve the quality of hair coat. Thus, as the extension of the trapping, fur farming of animals prized for their fur began. The critical change of the animal natural habitat and a feeding mode had consequently led to the domestication of fur animals. However, the knowledge on physiology and behavioural patterns of the population living in the wild proves to be valuable in order to maintain high standard of animal welfare. Only the optimal breeding conditions ensure obtaining high-quality final products (Gliński and Kostro, 2003; Kidd, 2008; Nowakowicz-Dębek and Łopuszyński, 2004, Nowakowicz-Dębek and Wlazło, 2011; Rucińska et al., 2005).

In some countries, fur animals are considered an important indicator species for monitoring environmental pollution. Responses of fur animals to environmental contaminants often resemble those observed in laboratory animals or even humans (Kidd, 2008). Special attention was given to minks as they feed on prey at higher trophic levels, including both, aquatic and terrestrial environments. Mink is a sensitive indicator to organic pollutants – dioxins or polychlorinated biphenyls (PCBs) present in the environments (Haynes et al. 2007; Bursian et al., 2006). Exposure to these substances manifests as lesions histologically detected in the maxilla and mandible of mink. According to Haynes et al. (2007), persistent exposure contributes to progression of the recognized lesions. Different diets fed to the farmed and free-living animals give rise not only to behavioural changes but physiological and biochemical as well displayed in the blood picture. The biochemical parameters of blood are valuable diagnostic tools applicable for assessment of animal body conditions, health or environmental conditions. They constitute substantial information on the mechanisms of organism functioning at many levels (Bursian et al. 2006). Differentiation of the environments, where both farmed and wild animals stay, contributes to variation in blood parameters (Damgaard et al., 2012; Nowakowicz-Dębek et al., 2013, Gliński and Kostro, 2003). Analysis of biochemical blood parameters allows detecting possible health problems of animals or even farm mismanagement (Gliński and Kostro, 2003). The objective of the studies was to compare chosen biochemical parameters in the blood of the farmed and wild minks, foxes and raccoon dogs.

Material and methods. The studies included carnivorous fur animals, i.e. red foxes, raccoon dogs and standard minks housed in the farm at the end of production cycle. The study was approved by the local research ethics committee in Lublin, Poland. Wild foxes, raccoons and minks of dark brown pelage were captured and maintained at the same farm for one month. The number of captured free-living animals resulted from small numbers of individuals in the population in the environment during the research period. The farm was situated at the rural area of south-eastern Poland. Its

environmental and hygienic conditions were regarded as good. All the animals were kept at the same environmental conditions, i.e. wire cages in open area under the roof. Both, farmed and wild animals were fed the same diet appropriate for a species and feeding stage. There was provided a diet formulated according to the recommended nutritional norms to that species. The basic diet components were as follows: poultry slaughter by-products – 50%, then beef by-products – 10%, MDM (Mechanically Deboned Meat) turkey meat – 5%, code fish by-products – 5%, meat and bone meal – 2%, feather meal – 2%, whole dried blood – 1%. The fodder was preserved with sodium pyrosulfate (2 kg/1,000 kg of fodder). The rest of the diet comprised cereal grains, sugar-beetroot pulp and commercial vitamin-mineral premixes (GUYOFOX PLUS 0.1%) supplemented according to the producer indications. The access to tap water was provided ad libitum (Gugołek, 2011, Nowakowicz-Dębek et al. 2013). Standard preventive measures relevant to each animal species were implemented at the farm. In all the treatment groups, the research material was collected from males of a similar age, in the IV feeding period (the turn of December/January). The first blood collection was performed after the one month acclimatization time of wild animals and the other one after the 30-days stay under the farm conditions. All the animals had blood taken at the same date. Blood was collected into commercial plastic tubes containing K_2EDTA (dipotassium ethylenediaminetetraacetic acid), (vacutainer Sarstedt, Poland) and delivered to the laboratory. Then, the blood samples were centrifuged and analyzed in a MINDRAY BS-130 (Mindray Building) chemistry analyzer. Blood plasma was examined to measure the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (BiLT), urea (UREA), lactate dehydrogenase (LDH) using Cormay monotests.

Statistical evaluation. The obtained results were analyzed with SAS v. 9.4 (SAS, 2013) statistical software package and analysis of variance. The means denoted with different letters a, b differ significantly at $P \leq 0.05$. The size of the groups is presented in Tables.

Results and discussion. Domestication alters not only animal behaviour but affects numerous physiological processes as well. Some changes are the result of intentional breeding efforts in an attempt to eliminate an undesirable trait of animal that decreases its usability and breeding value. Importantly, artificial selection can frequently produce the unfavourable correlated responses because of the genetic correlation between the traits. Meanwhile, an uncontrolled human-induced selection in the wild can cause irreversible phenotypic changes within the harvested populations and the environment (Araki et al., 2007; Allendorf and Hard, 2009; Tamlin et al., 2009). Blood examination proves to be an indispensable diagnostic tool to assess the health state of the populations under study and to indicate the exposure to local environmental pollutants (Person et al., 2012).

The results of blood biochemical parameters analysis of free-living and farm-raised raccoon dogs are summarized in Table 1. Statistically significant differences ($P \leq 0.05$) between the groups were determined for AST, ALT and LDH. The studied parameters indirectly depict living conditions of animals, their nutrition and the state of environment and at the same time, they reflect animal body condition. The free-living raccoon dogs showed lower values of ALT, AST and BiLT compared to the farmed ones. Statistically higher values were obtained for LDH. The parameters under investigation, except for LDH, were found within the limits presented by the Chinese authors for this group of furbearing animals (Ping et al., 2011).

Table 1. **Average values of blood biochemical parameters of free-living and farmed raccoon dogs** (n = 6 free-living /10 farm)

Parameter	Unit	Genre	Mean	Min	Max	Std
ALT	U/L	free-living	58.33 a	42.00	78.00	14.50
		breeding	76.33 b	57.00	92.00	12.74
AST	U/L	free-living	38.83 a	30.00	53.00	9.99
		breeding	57.83 b	46.00	84.00	14.44
BilT	µmol/l	free-living	0.188	0.017	0.684	0.256
		breeding	1.197	0.017	3.42	1.334
UREA	mmol/l	free-living	5.72	4.86	7.52	0.98
		breeding	5.62	4.15	7.14	1.20
LDH	U/L	free-living	320.17 a	251.00	417.00	61.48
		breeding	220.67 b	130.00	310.00	62.46
Average with different letters a, b, differ significantly at $P \leq 0.05$						

Despite the fact that raccoons and foxes belong to the same family *Canidae*, the data presented in Tables 1 and 2 indicate significant differences between the studied groups of animals. According to Ping et al., (2011), the fact that biochemical parameters determined for the raccoon dogs and foxes have different values is the evidence of the substantial differences between the species of animals. The mean values of ALT, AST, UREA summarized in Table 2 were higher in the wild foxes, while the bilirubin content and LDH activity level in the farmed ones. Ping et al., (2011) gave similar ranges of values for most of the parameters described, whereas

Kidd et al., (2009) reported slightly lower than those obtained in the present research, except for LDH. The activity level established was relatively higher than that reported by the mentioned authors. Most of the research results have been statistically significant (P≤0.05). Enhanced LDH activity is often considered to be a sequel of, among others, cell apoptosis, increased ischemic cellular membrane permeability, especially under exposure to toxins. Hence, comparing the values of each parameter should involve thorough consideration of organ-specific expression of enzymes (Kozhevnikova et al., 2004; Tiutiunnik et al., 2002).

Table 2. **Average values of blood biochemical parameters of free-living and farmed foxes** (n = 5 free-living/10 farm)

Parameter	Unit	Genre	Mean	Min	Max	Std
ALT	U/L	free-living	164.83 a	130.00	198.00	26.46
		breeding	88.89 b	61.00	131.00	23.71
AST	U/L	free-living	117.50 a	91.00	157.00	22.94
		breeding	48.89 b	32.00	62.00	11.57
BilT	µmol/l	free-living	7.832 a	2.736	13.66	4.087
		breeding	16.091 b	12.312	19.15	2.394
UREA	mmol/l	free-living	7.21 a	4.57	13.38	3.16
		breeding	4.78 b	4.00	5.51	0.44
LDH	U/L	free-living	529.00	330.00	772.00	190.84
		breeding	654.89	426.00	1093.0	211.26
Average with different letters a, b, differ significantly at P ≤ 0.05						

Table 3. **Average values of blood biochemical parameters of free-living and farmed minks** (n = 7 free-living /10 farm)

Parameter	Unit	Genre	Mean	Min	Max	Std
ALT	U/L	free-living	160.86 a	106.00	242.00	43.11
		breeding	113.29 b	97.00	143.00	15.82
AST	U/L	free-living	113.57 a	95.00	149.00	20.92
		breeding	77.43 b	64.00	99.00	13.11
BilT	µmol/l	free-living	2.035	0.171	2.907	1.267
		breeding	2.50	0.684	3.078	0.855
UREA	mmol/l	free-living	4.94	3.44	7.17	1.22
		breeding	4.24	2.72	5.76	1.01
LDH	U/L	free-living	1036.1 a	695.00	1408.0	259.12
		breeding	1410.17 b	1010.0	1813	305.9
Average with different letters a, b, differ significantly at P ≤ 0.05						

The results of blood biochemical evaluation of free-living and farmed minks are presented in Table 3. The analysis performed showed that the farmed minks had ALT activity level within the reference range given by Hunter (1996), while in the free-living minks far higher values were determined as against the farmed ones. The peak ALT activity level in the free-living minks exceeded the highest values reported for the lactating mink dams (norm 210 U/L), (Hunter, 1996).

The activity of the analyzed transaminases in the farmed and free-living animals was consistent with the results reported by Mikniene et al., (2010). The authors obtained the AST activity for males in the range of 76.30–166.00 U/l, while ALT within the 76.60–212.60U/l interval. Average AST level in both studied groups was found within the reference range, in spite of the markedly higher values obtained for the free-living animals. The exception was made by maximum AST activity recorded for the wild minks that surpassed the values presented by Hunter (1996). The values of the parameters, though, were consistent with the results of Damgaard et al., (2012) who determined ALT activity in minks on a restricted diet. Elevated AST and ALT activity and their ratio in blood plasma are regarded a sensitive indicator of hepatic abnormalities and precedes morphological markers of hepatocyte injury. A concurrent increase of AST, LDH, amylase and GGT (gamma-glutamyltransferase) activity is a helpful diagnostic assay for the pancreas, kidneys or inflamed intestinal mucosa. Transaminases are enzymes normally found in the liver parenchymal cells and, importantly, plasma transaminase elevation is known to be a very sensitive indicator of these cells injury (Meyer and Harley, 2013; Kozhevnikova, 2004; Schuster et al., 2003; Tiutiunnik et al., 2002; Tyopponen et al., 1982).

Urea is the major nitrogen product of protein metabolism which is excreted from the bloodstream through the kidneys. Therefore, a raised blood urine

nitrogen level indicates impaired renal function (Bis-Wencel et al., 2006; Tauson et al., 2001). Urea content in free-living and farmed minks was at a similar level (Table 3). The obtained values appeared to be slightly lower than those presented by Damgaard et al. (2012) but nearing those reported by Bis-Wencel et al., 2006; Tauson et al., 2001; Tauson and Wamberg, 1998) for minks fed a restricted diet. The average urea level in both groups under investigation fell within the reference interval set for this animal species. Differences were not significant statistically. Diagnostic value of this parameter is rather unreliable for carnivorous animals and the interpretation challenging because of its high variation, subject to different levels of dietary protein supply (Tauson and Wamberg, 1998).

Bilirubin is a metabolite of metalloporphyrins, especially hemoglobin, produced in the liver, spleen and bone marrow. The increased amounts of bilirubin may be associated with, among others, viral or toxic liver injury (Bis-Wencel et al., 2006). In the present study, the bilirubin concentration in the farmed minks reached 2.50 µmol/l and was slightly higher than the level established for free-living animals (Table 3). However, the values were lower as compared to those reported by Bis-Wencel et al. (2006). Average LDH activity was high in both treatment groups and the values statistically significant. The high values may prove a severe injury of cells (tissues) that consequently brings on a significant increase in plasma LDH. The farmed mink population managed under the standard farm conditions is characterized by low environmental variation and thus, the research results from the studies on these animals show low variation as well (Kidd et al., 2009).

The differences noted between the biochemical parameter values in the blood of free-living and farmed animals indicate adaptive separateness of these animals. Although the housing conditions and feeding habits were unified, considerable differences between the studied parameters were determined during the relatively short research period. The study results of chosen biochemical blood parameters of the wild population can add great practical value to efforts ensuring the welfare of farmed animals and to obtain optimum performance of foxes, raccoon dogs and minks.

Conclusions

1. The differences in the analyzed biochemical blood parameters of free-living animals and those under the farm conditions were shown.
2. Statistically significant higher LDH values were observed in free-living raccoon dogs ($P \leq 0.05$).
3. ALT, AST activity and UREA level ($P \leq 0.05$) were found to be significantly higher in free-living foxes, while bilirubin level in the farmed animals.
4. Significantly higher activity of AST, ALT was determined in free-living minks, whereas LDH in the farmed animals.

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