
ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXIII, 37

SECTIO EE

2005

Laboratory of Reproduction Biology of the Department of Animal and Environment Hygiene
Faculty of Biology and Animal Breeding, UA in Lublin,
Department of Toxicology, Faculty of Biology and Animal Breeding, UA in Lublin

HANNA BIS-WENCEL, LEON SABA, ADAM LICZMAŃSKI,
BOŻENA NOWAKOWICZ-DĘBEK

*The Level of Some Indices of the Oxidation State
in Blood Plasma of Mink at Slaughter Period Under
the Definite Maintenance and Feeding Conditions**

Poziom wybranych wskaźników stanu oksydacyjnego w osoczu krwi norek
w okresie uboju w określonych warunkach utrzymania i żywienia

Excess of aerobic radicals is hazardous for animal health owing to their high nonspecific reactivity. This may lead to the imbalance between antioxidants and prooxidants in favour of oxidation, that in a consequence induces the status called the oxidation stress. Numerous clinical examinations confirm the relation between the oxidation stress and health state disturbances [4, 8, 9]. The state mentioned above need not be caused by the direct influence of free radicals still it is of great importance in the whole chain of events giving rise to a polyeti-ological disease and development of concurrent symptoms. Thus, the sources of free radicals seem to be vital as they are developed in organism under the endo- and exogenous conditions. The latter include, among others, nutrition, ultraviolet radiation and substances polluting the environment predominantly [1, 2].

The objective of the present paper was to determine the values of some blood plasma markers considered oxidation state indices at minks aged 1 year, maintained in farm conditions.

MATERIAL AND METHODS

The studies were carried out at "C" farm, situated in the south-eastern part of Poland. The object was surrounded with a broad green belt, constituting a natural barrier for odours not to spread

*This work was conducted as part of the research project №. PO6Z 0512 25 financed by the State Committee for Scientific Research (2003–05)

away. The stock of the basic pack was around 500 females. The feed was supplemented with vitamin-mineral premix Guyofox at a dose 1.0 kg/t of ready feed. Till October an antioxidant additive sodium pyrosulphite was used at a dose making 0.2–0.3% of ready feed mass (table 1.). Throughout the experimental period the feed was provided *ad libitum*, with permanent water access.

The yearlings chosen for the examination were meant for slaughter. Blood was collected from the heart of a mink, of a fine variety "scan brown". In plasma there were determined: (GSH-GSSG) total glutathione concentration according to Akerboom and Sies' method, [1], (EC-SOD) activity of superoxide dismutase after the adrenaline method of Misra [1], (GPx) glutathione peroxidase activity, (TAS) total antioxidant status with a diagnostic test of RANDOX, and a level of soluble protein with L o w r y's method [1].

The determination mentioned above were performed with a two-beam spectrophotometer CE 7200 CECIL. The obtained results were analysed statistically, using the ANCOVA method.

RESULTS AND DISCUSSION

Glutathione (GSH+GSSG) belongs to the antioxidants operating on the basis of the non-enzymatic mechanism. A glutathione thiole group readily reacts with free radicals, the fastest with hydroxyl radical, and a bit slower with organic radicals, occurring at the aqueous phase. The reactions of glutathione and free radicals of organic substances, in particular proteins, may result in the "repair" of these particles, in favour of the formation of glutathione free radical [1]. The level of mean values (GSH+GSSG) at the examined minks developed within 0.110–0.240 U/l interval (table 2).

A vital role in the defensive system against the FR attack was performed by the antioxidative enzymes. They include, among others, superoxide dismutase (EC-SOD) and glutathione peroxidase (GPx). The enzymes mentioned above metabolize free radicals (O_2 in the case of SOD) or the half products (H_2O_2) in the case of (GPx) into less toxic or nontoxic products. The activity of superoxide dismutase in the extracellular fluids is lower than in intracellular ones. Despite this, the cell surface is protected, against superoxide anionoradical by means of small quantity of EC-SOD bounded with them [1]. Throughout the studies, the EC-SOD level reached 25.08–10.23 U/l. Glutathione peroxidase (GPx) is an adaptative enzyme; its activity increases in response to the oxidation stress with glutathione reductase [10]. It catalyzes the hydrogen peroxide reduction and organic peroxides by the reduced glutathione. The mean levels of glutathione peroxidase in minks ranged from 20 575.10–18 360.45 U/l and glutathione reductase activity mean levels were 97.45–108.70 U/l.

The response to the oxidation stress manifests itself with the definite anti-oxidation state that can be presented as the total activity (TAS). It is likely to be a perfect marker of this state as there are numerous interactions recorded be-

Table 1. The quantitative composition of feed supplied to minks and energy value of 1 kg

Product	01.08-15.09.	16.09-10.11.	11.11 for slaughter
	%	%	%
Cod	4.0	7.0	5.0
Sprat x	15.0	-	-
Poultry mixtures	42.0	50.0	55.0
Horses stomach	10.0	10.0	13.0
Meat-bone meal	8.0	8.0	6.0
Animal fat	3.0	2.0	1.0
Wheat meal – crude	9.6	9.6	12.0
Bran	2.0	2.0	2.0
Potatos boiled	-	5.0	-
Water	6.0	6.0	6.0
Guyofox	0,1	0,1	0,1
Sodium pyrosulphite	0.28	0.28	-
Rendox	0.02	0.02	-
The energy value Kcal/kg			
	1700	1670	1690
% EM			
Protein	35,1	33,3	33,0
Fat	52,4	52,5	52,2
Carbohydrates (CH ₂ O)	12,5	14,2	14,8

COD – fillet scraps (heads, backs)

SPRAT X-all fish

POULTRY MIXTURES – heads, paws, bowels

Table 2. The mean values of the blood oxidation stress parameters of mink N = 60

Parameters	Collection I	Collection II	Collection III
Glutathione peroxidase(GPx) U/l	20575.10	18706.60	18360.00
Glutathione Reductase U/l	99.70	108.70	97.45
Superoxide Dismutase EC-SOD U/l	20.050	10.230**	25.08
Total Glutathione GSH-GSSG U/l	0.205	0.110 *	0.240
Total Antioxidant Status (TAS) U/l	0.609	0.555	0.568
Protein g/l	78.80	85.10	80.75

*Significant differences between the groups, means noted with different letters are different significantly at * (p<0.05), ** at (p<0.01)

tween the antioxidants that are easily overlooked being individually determined. What is more, it is impossible to determine all the antioxidants because a part of them have not been identified so far. The total oxidative activity of minks oscillated at the level of 0.609 – 0.555 U/l. The mean values of soluble protein ranged 78.80 – 85.10 mg/ml.

Glutathione participates in the reconstitution of the damaged cell components. It is noteworthy that its main function is to keep protein thiole groups reduced, which in many cases is simply essential for the functional activity of proteins. What seems interesting is the fact that its concentration drop to only half of the values regarded the references, as a rule does not lead to any noticeable physiological effects. What is more, the enzymes interacting with GSH in a cell do not change their activity even under the conditions of a significant decrease of this tripeptide. It is only a considerable fall of GSH concentration, which reduces their efficiency [1]. In the carnivorous furry animals breeding, including minks, there appears a special risk of animal organism, exposure to the oxidation stress resulting from air pollution. It follows from the fact that furry animal farms emit some tens of odourforming substances, mainly sulphoorganic compounds, ketones, aldehydes, alcohols as well as aliphatic and aromatic hydrocarbons [6, 8, 9].

These compounds are characterized not only by noxious smell, but quite frequently these are toxic or carcinogenic substances for man and animal. It often happens that EC-SOD is termed a "locally specific" protective enzyme. It is connected with the analogy to the local specific development of radical OH [1, 7].

In human cells there was detected appearance of four different forms of glutathione peroxidase. The first one is intracellular called classical (CGPx). It occurs in different cells, among others in erythrocytes and its function is the protection of cells against the oxidation stress, especially hydrogen peroxide. The next form is GI-GPx, i.e. gastrointestinal peroxidase recorded in the alimentary tract walls [3]. Besides, its presence is also detected in the liver and some lines of neoplastic cells. It makes the barrier against the peroxides and xenobiotics that entered the alimentary tract. The volatile gaseous substances are generated at a farm, not only during the complex digestive processes recording in the stomach. They are also made due to intricate decomposition processes in discharges falling down or surged under the cages. Moreover, what seems important is the impact of high-energy and high-protein feed like, gurry or meat scraps [5,6]. A plasma form (PGPx) occurs mainly in the extracellular fluids and in tissues which are in contact with them (kidneys, placenta). The last form is glutathione peroxidase of phospholipide hydroxides (PHGPx). The greatest amount of this enzyme in mammals is detected in male testes. It is interesting that occurring in the sperm cell mitochondria, it is responsible for nearly half of the total protein content of external mitochondrial membrane of sperm, while it is inactive in mature sperm cells. No doubt it has a significant physiological function there as in the sperm cells of infertile man substantially smaller quantity of this enzyme is detected [1].

Owing to a lack of publications with the reference values, the values enclosed may be treated as preliminary studies.

CONCLUSION

1. Most of the analyzed markers were on similar levels.
2. The level of Superoxide Dismutase and the level of Total Glutathione indicated significant differences among the collections.

REFERENCES

1. Bartosz G.: The other face of oxygen. PWN, Warszawa 2003.
2. Benzie I. F. F., Strain J. J.: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239, 70–76, 1996.
3. Brigelius-Flohe R., Muller C., Menard J., Florian S., Schmehl K., Winkler K.: Functions of GI-GPx: lessons from selenium – dependent expression and intracellular localization. *Biofactors*, 14, 101–106, 2001.
4. Kleczkowski M., Kluciński W., Sitarska E., Sikora J.: The oxidation stress and some indices of the animal oxidation state. *Med. Wet.* 54, 166–171, 1998.
5. Nowakowicz-Dębek B., Saba L., Bis-Wencel H.: Emissionen aus Pelztierfarmen in Polen. *Agrarforschung*, 7 (2), 2000.
6. Nowakowicz-Dębek B., Saba L., Bis-Wencel H.: Zanieczyszczenie powietrza alkoholami, aldehydami i ketonami przez fermę zwierząt futerkowych. *Med. Wet.*, 57 (5), 346–348, 2001.
7. Makurland S. L.: Ceruloplasmin, extracellular-superoxide dismutase, and scavenging of superoxide anion radicals. *J. Free Rad. Biol. Med.*, 2, 255–260, 1986.
8. Saba L., Bis-Wencel H., Sławoń J., Polonis A.: Air and soil pollution by carnivorous furry animal farms. *Annales UMCS, sect. DD, vol. XI*, 215–222, 1993.
9. Saba L., Sławoń J., Tymczyńska L., Bis-Wencel H.: Sulphoorganic odour emission from carnivorous furry animal farms. *Annales UMCS, sec. DD, vol. XIV*, 177, 1996.
10. Sies H.: Oxidative Stress: introductory remarks. In: H. Sies (ed): *Oxidative stress*, Academic Press, 1–8, London 1985.

STRESZCZENIE

Nadmiar rodników tlenowych jest niebezpieczny dla zdrowia zwierząt ze względu na wysoką niespecyficzną ich reaktywność. Może doprowadzić to do zachwiania równowagi między antyoksydantami a prooksydantami na rzecz utlenienia, co w konsekwencji powoduje pojawienie się stanu zwanego stresem oksydacyjnym. Celem pracy było wyznaczenie wielkości wybranych parametrów osocza krwi, uznawanych za markery stanu antyoksydacyjnego, u norek w wieku jednego roku, utrzymywanych w określonych warunkach fermowych. Norki żywione były zgod-

nie z zaleceniami obowiązujących norm. Skład dawki pokarmowej uzależniony był od wieku i stanu fizjologicznego. Do karmy dodawano przeciwutleniacz Rendox w ilości 0,2l/tonę karmy oraz konserwant pirosiarczyn sodu w ilości 0,28% masy karmy. Karmę podawano do poszczególnych klatek raz dziennie. Zwierzęta jednoroczne wytypowane do doświadczenia przeznaczone były do uboju. Krew pobrano trzy razy, z serca, od 60 nerek odmiany barwnej scan brown. W osoczu oznaczono: całkowite stężenie glutationu (GSH-GSSG) wg metody Akerbooma i Siesa, aktywność dysmutazy ponadtlenukowej (EC-SOD) metodą adrenalinową wg Misra, aktywność peroksydazy glutationowej (GPx) i całkowitą aktywność oksydacyjną Total Antioxidant Status (TAS) testem diagnostycznym firmy RANDOX, poziom białka rozpuszczalnego metodą Lowrego. Powyższe oznaczenia wykonano przy użyciu spektrofotometru dwuwiązkowego CE 7200 firmy CECIL.