Praca oryginalna *Original paper*

Influence of different forms of selenium supplementation on the oxidant balance in the rat liver

IRENA MUSIK, MAŁGORZATA KIEŁCZYKOWSKA

Chair and Department of Medical Chemistry, I Medical Faculty with Dentistry Division, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland

Musik I., Kiełczykowska M.

Influence of different forms of selenium supplementation on the oxidant balance in the rat liver

Summary

The aim of this study was to estimate the influence of the administration of two selenoorganic compounds of different structures, as well as inorganic sodium selenite, on antioxidant parameters and lipid peroxidation in rat liver tissue. Adolescent male Wistar rats were treated through a stomach tube with saline (control), Na_2SeO_3 (group II), 4-(o-tolyl-)-selenosemicarbazide of 2-chlorobenzoic acid (chain structure – group III), 3-(2-chlorobenzoylamino-)-2-(o-tolylimino-)-4-methyl-4-selenazoline (ring structure – group IV) at a dose of $5 \cdot 10^{-4}$ mg of Se g^{-1} of b.w. once a day for a period of 10 days. Liver homogenates were examined to determine total antioxidant status (TAS), the activities of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx), the concentrations of ascorbic acid (AA) and reduced glutathione (GSH), as well as the concentration of malondialdehyde (MDA). TAS was significantly reduced in animals receiving selenoorganic compounds vs. control. SOD was unchanged and GPx decreased in all groups undergoing Se-administration vs. control. AA was decreased in group IV vs. both control and group II. GSH was unaltered vs. control in rats receiving selenocompounds. MDA was significantly decreased in group IV in comparison with all other groups. Selenium supplementation generally caused impairment of selected elements of antioxidant barrier, but the ring selenoorganic significantly decreased the lipid peroxidation level. Further studies with the use of diverse doses and longer supplementation periods, including studies concerning the action of selected selenoorganic compounds in pathological states, are needed to evaluate the usefulness of these substances as Se-supplements.

Keywords: selenium supplementation, antioxidant barrier, rats, liver

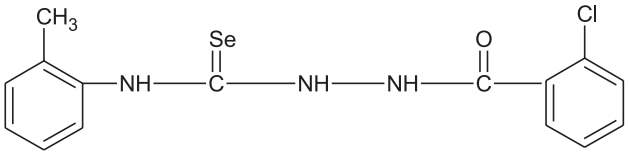
Selenium is an important microelement, and there have been many studies concerning its influence on metabolism (4, 5, 11, 22). They have revealed that its deficiency is connected with the occurrence of various diseases (19, 28) and that selenium treatment may cause beneficial effects in pathological states (8, 15, 25). However, the question of the optimal form and dosage of supplementation still remains unsolved, as selenium has a narrow margin between therapeutic and toxic levels, and its excess may be harmful (11, 22). For these reasons, numerous investigations have been performed, involving both inorganic and organic compounds, in order to identify the best supplements (1, 2, 14, 18, 18), but the results have not been fully satisfactory. Among other things, the relationships between selenium administration and the oxidative balance in the organism have been studied (4, 12, 16, 29). Ebselen, a ring selenoorganic compound of isoselenazole structure, has been found to possess antioxidant properties (7). Some organic selenium compounds of chain structure have also shown influence on antioxidative processes (4, 18). Oxidative stress – a state resulting from an increased generation of reactive oxygen species (ROS) – is believed to contribute to a severe damage to the organism and be involved into the pathogenesis of many illnesses (7, 24). ROS generated in the organism may cause lipid peroxidation, that is, the oxidation of lipid structures which leads to injury of cellular membranes (24). The organisms' defence against oxidative stress consists of a complex system of low- and high-molecule substances called the antioxidant barrier (2). Selenium is regarded as an antioxidant because it is a constituent of an antioxidative enzyme, glutathione peroxidase (GPx) (5, 10, 14). GPx and another antioxidant enzyme, superoxide dismutase (SOD), belong to the main high-molecule antioxidants,

whereas reduced glutathione (GSH) and ascorbic acid (AA) are important low-molecule antioxidants.

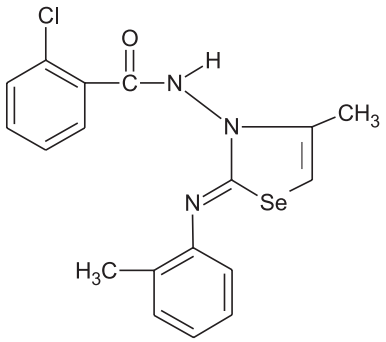
To contribute to the research on the best form and safe dosage of selenium supplementation, we studied the effect of different Se forms on the oxidant balance in the rat liver. Various organic compounds of ring formulas have already been investigated, both *in vivo* and *in vitro*, as have been derivatives of chain structure (4, 15, 17, 18, 21), whereas sodium selenite, an acknowledged inorganic Se-supplement, is still used in clinical practice (25) and as a supplement of animal food (20). The present study included two newly synthesized organic selenocompounds of different structures (ring and chain), and compared their influence with that of sodium selenite.

Material and methods

Two selenoorganic compounds were synthesized in the present study: compound A (chain structure) 4-(o-tolyl-)--selenosemicarbazide of 2-chlorobenzoic acid (16) and compound B (ring structure) 3-(2-chlorobenzoylamino-)--2-(o-tolylimino-)-4-methyl-4-selenazoline (18) (Fig. 1).



compound A



compound B

Fig 1.

The experiment was performed on adolescent male Wistar rats (110-150 g body weight). After an acclimatization period of three days, the animals were randomly divided into four groups (ten animals in each): group I (control), treated with saline; group II (Na_2SeO_3), treated with sodium selenite; group III (Se-chain), treated with compound A; and group IV (Se-ring), treated with compound B. Sodium selenite was given in the form of water solution. Organic compounds given to groups III and IV were suspended in emulsion composed of oil, gum arabic, and water in the following proportion 2 : 1 : 1.5. The administration was performed with a stomach tube. Selenium compounds were given to rats at a dose of $5 \cdot 10^{-4}$ mg of Se g^{-1} of b.w. once a day for a period of 10 days. Body weight of each animal was measured every day before Se-administration, and the appropriate amount of selenium compound was calculated. The rats had free access to standard feed LSM and drinking water. The study was performed according to statutory bioethical standards and approved by the First Local Ethical Commission of the Medical University of Lublin, acceptance no. 65/AM/2004.

After the end of the experiment, the animals were sacrificed under pentothal narcosis, and samples of liver tissue were collected. Ten per cent (w/v) tissue homogenates were prepared in 0.1 mol dm^{-3} Tris-HCl buffer, pH = 7.4. Supernatants were obtained by centrifugation at 5000 × g for 30 min. The following substances were determined in liver homogenates: total antioxidant status (TAS), the activities of SOD and GPx, and the concentrations of AA, GSH, and the lipid peroxidation marker – malondialdehyde (MDA). TAS was measured with a diagnostic kit produced by RANDOX and expressed in mmol $\cdot$ g^{-1} of protein. SOD and GPx activities were determined with RANSOD and RANSEL diagnostic kits produced by RANDOX and expressed in U $\cdot$ mg^{-1} of protein and U $\cdot$ g^{-1} of protein, respectively. The GSH concentration was determined with a BIOXYTECH® GSH-400™ kit produced by OxisResearch™ and expressed in μg of GSH $\cdot$ mg^{-1} of protein. The AA concentration was determined by the modified Kyaw method and expressed in μmol of AA $\cdot$ g^{-1} of protein (23). The MDA concentration was determined by the Ledwożyw et al. (13) method and expressed in nmol of MDA $\cdot$ mg^{-1} of protein. Protein was measured by Bradford's method (3). The assays were performed with a SPECORD M40 spectrophotometer (Zeiss Jena).

Statistical analysis was performed with the ANOVA test. Comparisons between control and Se-supplemented groups, as well as between individual Se-supplemented groups, were made with Tukey's HSD test or Dunnett's T3 test. Values were considered significant with $p < 0.05$. A contrast analysis was also performed to evaluate the significance of differences between connected groups. The differences between group I and connected groups II + III + IV (selenium supplementation) as well as between group II (inorganic selenium) and connected groups III + IV (organic selenium) were estimated. Values were considered significant with $p < 0.05$.

Results and discussion

In the livers of rats undergoing Se supplementation, TAS was generally found to be decreased, although this effect was dependent on the form of administration. Inorganic sodium selenite depleted liver TAS insignificantly, whereas both organic compounds diminished it markedly in comparison with control.

SOD activity was unaltered in all groups receiving selenium supplements in comparison with control. Selenium administration decreased GPx activity vs. control regardless of the form of supplement. In the case of these three parameters, no differences between the groups receiving selenium were observed.

The effect of Se-compounds on AA concentration was strongly dependent on their structures. The greatest influence was observed in group IV, receiving the ring organic compound, in which a statistically significant depletion vs. control was found. In group II (Na_2SeO_3), inorganic form of Se caused an insignificant decrease. The chain selenocompound given to group III did not affect AA concentration. GSH concentration remained unaltered vs. control in all groups undergoing Se-supplementation, but in group IV (ring organic Se) a decrease in comparison with the Na_2SeO_3 group was observed.

Tab. 1. The enzymatic and non-enzymatic elements of the antioxidant barrier in the liver of rats receiving different Se-supplements ($\bar{x} \pm SD$)

Parameters studied	Group			
	I (control)	II (Na_2SeO_3)	III (Se-chain)	IV (Se-ring)
TAS (mmol • g^{-1} of protein)	0.33 ± 0.12	0.23 ± 0.05	0.14 ± 0.04*** (H)	0.14 ± 0.04*** (H)
SOD (U • mg^{-1} of protein)	29.82 ± 9.77	26.32 ± 11.10	25.48 ± 5.00	28.05 ± 12.80
GPx (U • g^{-1} of protein)	602 ± 141	329 ± 92* (D)	288 ± 79* (D)	237 ± 57** (D)
AA (µmol • g^{-1} of protein)	8.27 ± 2.58	5.64 ± 1.02	8.54 ± 1.46^{A} (D)	3.08 ± 0.75$^{*, A, Y}$ (D)
GSH (µg • mg^{-1} of protein)	21.46 ± 6.24	27.16 ± 2.39	21.53 ± 4.83	17.30 ± 4.66^{A} (H)
MDA (nmol • mg^{-1} of protein)	10.69 ± 4.26	9.20 ± 1.78	13.98 ± 3.45	4.35 ± 1.43$^{*, B, X}$ (D)

Explanations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. group I; A – $p < 0.05$, B – $p < 0.01$ vs. group II; X – $p < 0.05$, Y – $p < 0.01$ vs. group III; (H) Tukey's test HSD; (D) Dunnett's test T3

With regard to MDA concentration, the administration of inorganic Se resulted in no significant change vs. control. In group III (Se-chain), a slight, insignificant increase in MDA concentration was observed. The effect of supplementation was the greatest in group IV (Se-ring), in which a marked depression in comparison with control was found. All the outcomes obtained are collected in Tab. 1.

Tab. 2. Contrast analysis between group I (no selenium) and connected groups II + III + IV (selenium supplementation), as well as between group II (inorganic selenium) and connected groups III + IV (organic selenium)

Groups	Parameter					
	TAS	SOD	GPx	AA	MDA	GSH
II + III + IV vs. I	*** ↓	NS	*** ↓	** ↓	NS	
III + IV vs. II	* ↓	NS			NS	** ↓

Explanations: ↓ – decrease; * $p < 0.05$; **$p < 0.01$; *** $p < 0.001$; NS – no significant difference

The results of a contrast analysis are shown in Tab. 2. Selenium supplementation, regardless of its form, decreased TAS, GPx activity, and AA concentration in the rat liver. In comparison with the popular inorganic supplement Na_2SeO_3, organic selenium supplements diminished TAS and GSH concentration.

Se supplementation was found to decrease TAS values, particularly in animals receiving organic selenium. Relationships between Se intake and TAS in tissues have already been reported. A Se-deficient diet given to weanling male rats decreased total antioxidant capacity in tissues, especially in the liver and the kidneys, whereas selenium supplementation showed a slight ameliorating effect in the liver (28).

No changes in SOD or depletion of GPx were observed as a result of Se supplementation. A comparison of the available literature data shows that outcomes obtained by other authors are inconsistent. Hama et al. report that Se-enriched plants given to rats in diet caused a dose-dependent depletion of liver GPx (11). In pigs, dietary supplementation with sodium selenite or selenomethionine increased liver GPx activity (29). Cases et al. (4) observed that a Se-deficient diet caused a decrease in liver GPx activity in rats. In addition, the influence of three different selenium supplements (sodium selenite, selenomethionine, and Se-enriched algae *Spirulina*) on GPx activity was investigated, and the supplementation was found to result in GPx restoration, but inorganic selenite displayed the greatest efficacy. Furthermore, the difference between the effects of organic Se supplements was observed after 56 days of administration, and selenomethionine was found to be more beneficent. The two organoselenium compounds of different structures used in our experiment also differed slightly from each other, as well as from inorganic selenite, with regard to their influence on GPx activity. Similar outcomes concerning organic forms were observed in our previous study, carried out on mice (18). Na_2SeO_3 given to 3-month-old mice for 10 days at half the dose used in the present study caused a marked increase in liver GPx activity. Interestingly, the same experiment performed on 6-month-old mice resulted in no significant changes (5). In the study carried out on mice, an insignificant increase in liver GPx resulting from selenite administration was observed (18). In weanling male rats, dietary selenium deficit resulted in a decreased liver GPx, whereas SOD remained unaltered. Additional supplementation of Se-deficient animals in drinking water did not influence liver SOD, but significantly enhanced liver GPx (28). A single intraperitoneal injection of Na_2SeO_3 given to young rats fed a selenium-deficient diet caused a significant increase in GPx, but no change in SOD activity

in the liver (2). Also in the present study, no influence of selenium administration on liver SOD activity was observed. A study concerning relationships between the dietary level of sodium selenite and glutathione peroxidase showed that liver GPx activity was enhanced in a dose-dependent way up to a selenium level of 240 μg Se · kg^{-1} of diet. Further increase in Se content in food did not cause any distinct changes. However, the duration of that experiment was much longer: five weeks (26). A significant decrease in liver GPx activity observed in our study could be related to the relatively big dose and the short time of administration, which did not allow the organism to develop adaptive mechanisms.

In the present experiment, AA and GSH were not significantly altered vs. control, except the decrease in AA in group IV (Se-ring). Ognjanović et al. (19) found that sodium selenite increased AA in livers of both healthy and Cd-exposed rats, but in that case the same dose was administered almost three times longer. Another study showed no effect of selenite on glutathione concentration in the liver. Liver GSH in both healthy and Hg-exposed rats remained unchanged (1). Similarly, during the same period, supplementation with half the dose of selenium (Na_2SeO_3) used in our study caused no changes in liver total glutathione in 3- and 6-month-old mice (5). In rats receiving a calculi forming diet, additional supplementation with Na_2SeO_3 for 30 days enhanced both AA and GSH in liver tissue (24), but in that case the effect of selenium was related to the action of another factor, that is, a specific diet. An *in vitro* study performed by Pawlas and Małecki (21) on cortical neurons of rat fetuses treated with an organoselenium ring compound (ebselen) showed that GSH concentration increased up to a stipulated value of ebselen concentration. Higher doses caused no further enhancement of GSH. This suggests that GSH, similarly as GPx activity (26), increases in a Se-dose--dependent way until a kind of "saturation" occurs. These observations are consistent, considering the fact that reduced glutathione is a substrate for glutathione peroxidase. In the present experiment, a significant decrease in GPx activity in group IV (Se-ring) was accompanied by a slight, insignificant depletion of GSH concentration vs. control. The effect of Se supplementation on the lipid peroxidation level in this study depended on the Se-compound applied, which suggests that the effect of selenium on the organism depends on the form of administration. The available data concerning this question are quite divergent, but differences in the influence of various forms of selenium on MDA have also been reported. In broilers fed a diet containing Se, as Na_2SeO_3 or Se-enriched yeast, with the addition of vitamin E for 21 days, liver MDA concentration was lower in birds receiving yeast (14). In the livers of pigs fed a diet supplemented with Na_2SeO_3 or selenomethionine, MDA was decreased, but no statistical difference between inorganic and organic Se-form was noted (29). El-Demerdash (6) report that in rats, both healthy and Al-exposed, sodium selenite caused a significant depletion of lipid peroxidation in the liver. However, both the dose applied and the duration of the experiment (30 days) were different than in our study. Ognjanović et al. (19) report that lipid peroxides were unchanged in healthy rats, but significantly depressed in Cd-exposed ones, as a consequence of selenite administration. In contrast, Agarval and Behari (1) observed that sodium selenite administered for 20 days increased MDA concentration in the livers of both healthy and mercury--exposed rats.

Concluding, since selenium deficiency has been observed in many illnesses (9, 27), the importance of maintaining an adequate selenium level in the organism is undeniable (10). However, the question of the most efficient form of selenium is still open. In our experiment, short-term selenium supplementation generally caused impairment of selected elements of the antioxidant barrier, but the ring selenoorganic significantly decreased the lipid peroxidation level. Further studies with the use of diverse doses and longer supplementation periods, including studies concerning the action of selected selenoorganic compounds in pathological states, are needed to evaluate the usefulness of these substances as Se-supplements.

References

1. *Agarval R., Behari J. R.*: Effect of Selenium Pretreatment in Chronic Mercury Intoxication in Rats. Bull. Environ. Contam. Toxicol. 2007, 79, 306-310.
2. *Agay D., Sandre C., Ducros V., Faure H., Cruz C., Alonso A., Roussel A. M., Chancerelle Y.*: Optimization of selenium status by a single intraperitoneal injection of Se in Se-deficient rat: Possible application to burned patient treatment. Free Radic. Biol. Med. 2005, 39, 762-768.
3. *Bradford M. M.*: A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 1976, 72, 248-254.
4. *Cases J., Vacchina V., Napolitano A., Caporiccio B., Besançon P., Lobinski R., Rouanet J. M.*: Selenium from Selenium-Rich Spirulina is Less Bioavailable than Selenium from Sodium Selenite and Selenomethionine in Selenium--Deficient Rats. J. Nutr. 2001, 131, 2343-2350.
5. *Dolezych B., Szulinska E.*: Selenium modifies glutathione peroxidase activity and glutathione concentration in mice exposed to ozone-provoked oxidative stress. J. Trace Elem. Med. Biol. 2003, 17, 133-137.
6. *El-Demerdash F. M.*: Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J. Trace Elem. Med. Biol. 2004, 18, 113-121.
7. *Filipovska A., Kelso G. F., Brown S. E., Beer S. M., Smith R. A. J., Murphy M. P.*: Synthesis and Characterization of a Triphenylphosphonium-conjugated Peroxidase Mimetic. Insights into the Interaction of Ebselen with Mitochondria. J. Biol. Chem. 2005, 28, 24113-24126.
8. *Gosney M. A., Hammond M. F., Shenkin A., Allsup S.*: Effect of micronutrient supplementation on mood in nursing home residents. Gerontology 2008, 54, 292-299.
9. *Gromadzinska J., Wasowicz W., Rydzynski K., Szeszenia-Dabrowska N.*: Oxidative-Stress Markers in Blood of Lung Cancer Patients Occupationally Exposed to Carcinogens. Biol. Trace Elem. Res. 2003, 91, 203-215.
10. *Ha E. J., Smith A. M.*: Selenium-dependent Glutathione Peroxidase Activity is Increased in Healthy Post-Menopausal Women. Biol. Trace Elem. Res. 2009, 131, 90-95.
11. *Hama H., Yamanoshita O., Chiba M., Takeda I., Nakajima T.*: Selenium--enriched Japanese Radish Sprouts Influence Glutathione Peroxidase and Glutathione S-Transferase in an Organ-specific Manner in Rats. J. Occup. Health 2008, 50, 147-154.
12. *Juniper D. T., Phipps R. H., Bertin G.*: Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribu-

tion and meat quality in commercial-line turkeys. Animal 2011, 5, 1751--1760.
13. *Ledwożyw A., Michalak J., Stępień A., Kądziołka A.*: The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clin. Chim. Acta 1986, 155, 275--284.
14. *Marković R., Jovanović B. I., Baltić Ž. M., Šefer D., Petrujkić B., Sinovec Z.*: Effects of selenium supplementation as sodium selenite or selenized yeast and different amounts of vitamin E on selenium and vitamin E status of broilers. Acta Vet. (Beograd) 2008, 58, 369-380.
15. *Mazokopakis E. E., Papadakis J. A., Papadomanolaki M. G., Batistakis A. G., Giannakopoulos T. G., Protopapadakis E. E., Ganotakis E. S.*: Effects of 12 months treatment with L-selenomethionine on serum anti-TPO levels in patients with Hashimoto's thyroiditis. Thyroid 2007, 17, 609-612.
16. *Musik I., Kiełczykowska M., Hordyjewska A., Pasternak K.*: Influence of different forms of selenium supplementation on superoxide dismutase activity and total antioxidant status in rats. Ann. Univ. Mariae Curie Sklodowska Pharm. 2009, 22, 95-101.
17. *Musik I., Kozioł-Montewka M., Toś-Luty S., Donica H., Pasternak K., Wawrzycki S.*: Comparison of selenium distribution in mice organs after the supplementation with inorganic and organic selenium compound selenosemicarbazide. Ann. Univ. Mariae Curie Sklodowska Med. 2002, 57, 15-22.
18. *Musik I., Starosławska E., Pasternak K.*: Effect of organic selenium compounds on the activity of glutathione peroxidase and superoxide dismutase in selected mouse tissues. Bull. Vet. Inst. Pulawy 2003, 47, 567-573.
19. *Ognjanović B. I., Marković S. D., Pavlović S. Z., Žikić R. V., Štajn A. Š., Saičić Z. S.*: Effect of Chronic Cadmium Exposure on Antioxidant Defense System in Some Tissue of Rats: Protective Effect of Selenium. Physiol. Res. 2008, 57, 403-411.
20. *Pavlović Z., Miletić I., Jokić Ž., Pavlovski Z., Škrbić Z., Šobajić S.*: The Effect of Level and Source of Dietary Selenium Supplementation on Eggshell Quality. Biol. Trace Elem. Res. 2010, 133, 197-202.
21. *Pawlas N., Małecki A.*: Effects of ebselen on glutathione level in neurons exposed to arachidonic acid and 4-hydroxynonenal during stimulated ischemia in vitro. Pharmacol. Rep. 2007, 59, 708-714.
22. *Peyroche G., Saveanu C., Dauplais M., Lazard M., Beuneu F., Decourty L., Malabat C., Jacquier A., Blanquet S., Plateau P.*: Sodium selenide toxicity is mediated by O_2-dependent DNA breaks. PLoS One. 2012, 7, e36343.
23. *Rutkowski M., Grzegorczyk K.*: Kolorymetryczne oznaczanie stężenia witaminy C w osoczu krwi przy użyciu odczynnika fosforowolframowego – modyfikacja metody Kyaw. Diagn. Lab. 1998, 34, 511-520.
24. *Santhosh Kumar M., Selvam R.*: Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J. Nutr. Biochem. 2003, 14, 306-313.
25. *Schnabel R., Lubos E., Messow C. M., Sinning C. R., Zeller T., Wild P. S., Peetz D., Handy D. E., Munzel T., Loscalzo J., Lackner K. J., Blankenberg S.*: Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease The Selenium Therapy in Coronary Artery disease Patients (SETCAP) Study. Am. Heart J. 2008, 156, 1201.e1--1201.e11.
26. *Venardos K., Harrison G., Headrick J., Perkins A.*: Effects of dietary selenium on glutathione peroxidase and thioredoxin reductase activity and recovery from cardiac ischemia-reperfusion. J. Trace Elem. Med. Biol. 2004, 18, 81-88.
27. *Whiting P. H., Kalansooriya A., Holbrook I., Haddad F., Jennings P. E.*: The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus. Br. J. Biomed. Sci. 2008, 65, 71-74.
28. *Wu Q., Huang K.*: Effect of Long-Term Se Deficiency on the Antioxidants Capacities of Rat Vascular Tissue. Biol. Trace Elem. Res. 2004, 98, 73-84.
29. *Zhan X. A., Wang M., Zhao R. Q., Li W. F., Xu Z. R.*: Effects of different selenium source on selenium distribution, loin quality and antioxidants status in finishing pigs. Anim. Food Sci. Technol. 2007, 132, 202-211.

Corresponding author: Irena Musik, PhD, Chodźki 4a, 20-093 Lublin, Poland; e-mail: irena.musik@umlub.pl