Proceedings of the

4-7 july **2000** – Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910
(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

ERDÉLYI M., VIRÁG GY., MÉZES M.

EFFECT OF SUPRANUTRITIONAL ADDITIVE SELENIUM SUPPLY ON THE TISSUE SELENIUM CONCENTRATION AND THE ACTIVITY OF GLUTATHIONE PEROXIDASE ENZYME IN RABBIT

Volume C, pages 183-188
-

EFFECT OF SUPRANUTRITIONAL ADDITIVE SELENIUM SUPPLY ON THE TISSUE SELENIUM CONCENTRATION AND THE ACTIVITY OF GLUTATHIONE PEROXIDASE ENZYME IN RABBIT

ERDÉLYI, M.*, VIRÁG, GY.**#, MÉZES M.*
#

* Department of Nutrition, "Szent István" University, H-2103 Gödöllõ, Páter K. u. 1., Hungary
** Institute for Small Animal Breeding and Nutrition, H-2100, Gödöllõ, POBox.: 417., Hungary

ABSTRACT

Selenium is an essential element for animals. Among other prominent roles it is present in special proteins, like the selenium-dependent glutathione peroxidase enzymes. As these enzymes have selenium at the active site, their activity is supposed to correlate with the selenium status. Little is known about the effect of selenium supplementation on the tissue Se concentration and GSHPx activity. Therefore, we studied the selenium concentration in the blood and in liver, while enzyme activity was measured in several tissues of New Zealand White rabbit. Two groups of animals (6 individual each) were involved in the experiment. One group was fed basal diet (0.125 ppm), and selenium supplemented diet was provided for the other one (0.314 ppm). Selenium concentration of whole blood and liver was measured in both groups, and significantly higher Se-concentration was found in the blood of supplemented group (99.7 vs 70.6 μg/l, $P<0.01$). Enzyme activity was significantly decreased in the liver ($P<0.05$) and in the genital organs ($P<0.05$) by Se supplementation and a slight increase appeared in the pancreas, erythrocyte haemolysate and in the kidney. According to literature data, these data might be the result of an efficient process for the elimination of exceeding Se. It is also supposed that selenium binds the co-substrate of the enzyme, the reduced glutathione, in the form of selenodiglutathione, which inhibits enzyme action, but this has to be proven in future studies.

INTRODUCTION

Glutathione peroxidase (GSHPx) has crucial role in the antioxidant defence of the animals. The special characteristic of this enzyme is that it contains selenium at the active site. Therefore, selenium is supposed to play a predominant role in the enzyme activity and regulation.
Contrary to the case of selenium deficiency much less data are available on the effect of additive selenium supply and its relation to the glutathione peroxidase activity. There are results suggesting that enzyme activity increases significantly with dietary selenium supplementation in chickens (Arai et al., 1994). Correlation between enzyme activity and selenium supplementation is not first order type as enzyme activity seems to reach a plateau (Gaál, 1998). This plateau value was found to be 0.2 ppm Se/kg feed for swines for the cellular, plasma and phospholipid hydroperoxid isozyme forms (Lei et al., 1998).

Mechanism, through which selenium excess accomplishes its effect, is also not clear. It seems to depend on the form in which Se is added to the diet (Wolffram et al, 1985). Burk (1986) proposed that the availability of various selenium-containing compounds for incorporation to provide selenium for glutathione peroxidase varies owing to the differences in the pathway for liberation of selenium from dietary compounds into the intracellular selenium pool. In general it is declared that organic forms are better utilised, than the inorganic ones (Mahan et al, 1996). In an experiment of Mahan et al. (1996), sodium selenite was found to be more biologically available for glutathione peroxidase activity, than the selenium enriched yeast source, as the serum GSHPx activity plateau was reached at 0.1 and 0.3 ppm, respectively. Other authors present data suggesting that at low

To whom correspondence should be addressed: virag@sunserv.katki.hu

selenium levels selenite produces higher Se deposition and higher GSHPx activities, than high-Se yeast, while at a higher level this latter selenium source shows higher measures (Yoshida et al, 1999). Leist and co-workers (1999) have found difference in the cellular effect of different selenium forms. In an *in vitro* experiment selenite and selenocystein supplementation have increased cellular selenium uptake and also GSHPx enzymes were found to be induced, whereas selenium supplementation in the form of selenomethionine resulted in increased selenium uptake, as well, but with no effect on GSHPx activities (Leist et al, 1999).

In the presented experiment our aim was to investigate what differences occur in tissue Se concentration and glutathione peroxidase activity as a result of supranutritional organic Se supply, and which organs have the potential to overcome the effects of Se surplus.

MATERIALS AND METHODS

Animals and diets

Halfsib New Zealand white rabbits at 10-weeks-of-age with same body weight were divided into two groups (6 individuals in each group, 3 males and 3 females in both). Rabbits in the control group (C) were fed on basal, commercial broiler rabbit diet (dry matter: 877g/kg feed, crude protein 180g/kg DM, crude fiber 117.3 g/kg DM, crude fat: 90,2 g/kg DM, N-free extract: 557 g/kg DM) for five weeks. Rabbits in the experimental group (S) were fed on basal diet to which 0.3 ppm SelenoYeast (AllTech) was added before granulation. SelenoYeast is a organic selenium preparation with 1000 ppm selenium content according to producer's specification. Selenium content of the basal diet was 0.125 ppm, while the supplemented feed was measured to contain 0.314 ppm selenium. Diets were fed *ad libitum*. Rabbits at 16-weeks-of-age were slaughtered and *post mortem* sampling was done.

Collection of blood and preparation of tissue samples

Blood was taken from the cervical vascular complex into a tube with appropriate amount of EDTA. Plasma was separated with centrifugation (2500 rpm, 20 min). 1:9 rate haemolysate was prepared from the erythrocytes with distilled water. Liver, kidney, pancreas, genital organ (ovary or testis) and femoral muscle samples were obtained *post mortem*. All the samples were stored at -20 °C until measurements. Immediately before measurement, tissue samples were homogenised in 9 volumes normal saline solution with Teflon homogeniser, followed by centrifugation (10000 rpm, 30 min, at 4 °C). The resulted supernatant was used as tissue extract for the further measurements.

Selenium concentration assay

Selenium content of feed, blood and liver was measured with atom absorption flameless photometry (UNICAM 939 QZ AA spectrometer). For spectrometry the samples were prepared by ultrasound sonication with a mixture of nitric acid and hydrogen peroxide.

Glutathione peroxidase activity assay

Glutathione peroxidase (EC 1.11.1.9.) activity was measured with end-point direct assay (Matkovics et al., 1988). Basis of this method is the reduction of cumene-hydroperoxide in the presence of glutathione, and formation of a yellow end product with dithionitrobensoic acid (DTNB). Concentration dependent absorbency was measured at 412nm (FUJI spectrophotometer). Enzyme activity was expressed as unit per mg protein. Protein concentration was determined with Biuret method in blood plasma and erythrocyte homogenisate samples (RANDOX Total Protein

Kit), while Foline phenol reagent was used with tissue homogenisate supernatant samples (Lowry et al. 1951).

Statistics

Measurement data were analysed with Excel 5.0 software utilising two way ANOVA.

RESULTS AND DISCUSSION

Selenium concentration

Several literature data on feed Se concentration requirement of rabbit are available, but even the highest value is lower than 0.15 ppm, while the selenium content of our supplemented feed was 0.314 ppm. Average selenium intake, based upon the feed consumption, was 13.96 ± 0.5 and 4.36 ± 0.44 μg/kg body weight/day of the supplemented and the control animals, respectively. According to these data, selenium intake of the individuals in the supplemented group was much higher than the normal dietary level of 0.1 ppm (Mateos and Piquer, 1994) and only slightly lower than the maximum safe dietary selenium of 15 μg/kg body weight/day, reported by Whanger et al. (1996).
Whole blood and liver selenium concentrations were measured in the two groups, as well. Selenium concentration was higher in group S than in group C, both in the blood (+ 41,4 %) and in the liver (16,5 %), but the difference was significant ($P<0.01$) only for the blood (See Table 1.).

Table 1. Selenium concentration in the blood and in the liver

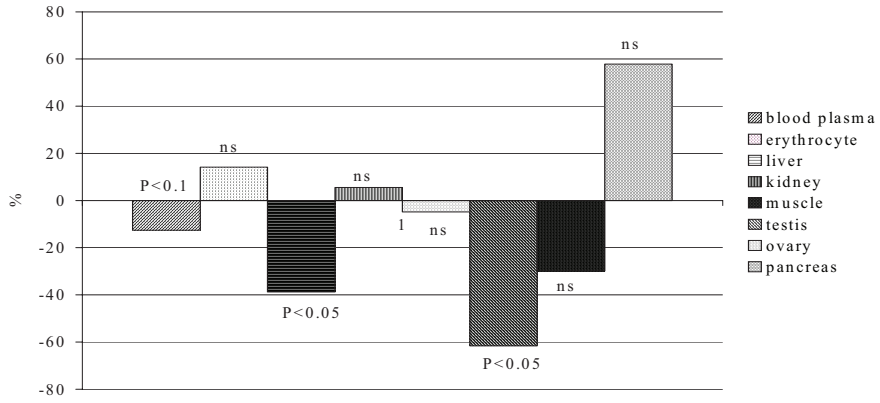
	Control group	Supplemented group
Whole Blood (μg/l)	$70.55^{A} \pm 8.3$	$99.7^{B} \pm 8.3$
Liver (μg /kg)	178.8 ± 135	208.4 ± 164

A, B: $P<0.01$

Enzyme activity

Enzyme activity changes in the tissue samples were different both in their tendency and extent. The possible reasons of this might be the different bio-availability of high selenium yeast for the different isozymes or the age and function related difference of the isoforms. In general, organic selenium compounds give rise to GSHPx activity, but the rate of change was different in the different tissues (Smith et al., 1974). Enzyme activity values measured in kidney, erythrocyte haemolysate and pancreas samples agree the former statement, as slight, but insignificant increase was found in the treated group. In the case of pancreas the activity change was found to be insignificant because the variance is high as a result of small animal number.
However, significant drop was measured in the enzyme activity in liver and testis samples, and the same, but not significant change was found in muscle, blood plasma and ovary (Figure 1).

Figure 1. Enzyme activity data in the different tissues (enzyme activity of different tissues in the supplemented group relatively to the values detected in the control group (%); Significance data refers to the activity data and not to the ratio.)



Discussion

The significantly higher blood selenium level in the supplemented group means high intestinal absorption level of the selenium enriched yeast culture, which is known to result in steady rise in the blood selenium concentration (Clausen and Nielsen, 1988).

The relatively small difference in the liver selenium concentration might be in accordance with the finding of Humaloja and Mykkänen, (1986). They showed that liver selenium level cannot be used as an indicator of the efficiency of selenium absorption in short-term studies, since after dosing the efficiency of selenomethionin accumulation is relatively low. This might be due to the higher urinary excretion of the selenium in selenium surplus proven earlier by Whanger et al., 1996, as the excreted form of the selenium is prepared in the liver.

Although literature data for rabbit tissue selenium concentration are not available, according to data of other livestock species, both whole blood and liver selenium levels in each group reflect slightly deficient selenium supply (Toyoda et al., 1988; Lingaas et al., 1990; Heimann et al., 1984, Avissar et al., 1994). A possible explanation of this finding might be the existence of low and high selenium responsive types of rabbits. This theory is based on the findings of Sankari and Atroshi (1983) in a Finnsheep population. If this theory is true our rabbits were belonged to the low selenium responsive type, and this was the reason of the relatively low selenium concentrations in the analyzed tissues. Another reason of this low selenium concentration is partly supposed to be high excretion. It is well known, that total body retention of the selenium supply is inversely related to the dietary selenium level (Burk et al., 1972). Bersényi et al. (1998) has shown that in rabbits 47.41% of the selenium intake is excreted in the urine and the feces.

According to activity data we do not know the real reasons of the changes, but it is known that excess selenium has the ability to form selenodiglutathione, what results in reduced glutathione concentration. As glutathione is a co-substrate of the GSHPx enzyme, the activity drop might be the consequence of the substrate deficiency. This is, the so called, prooxidant characteristic of selenium (Wahnger et al, 1996).

Altogether, as the supranutritional selenium did not caused drastic changes in selenium concentration of the blood and liver, as well as in enzyme activity it is proposed that animals have an appropriate mechanism to get rid of excess selenium (below toxic level). This mechanism would be rather important to describe as high dose selenium supplementation is found to be reasonable

solution for liver therapy in metabolic disorders (Cantor et al., 1982). Also good results were obtained in the selenium based treatment of mycotoxicosis (Shen et al, 1994).

***Acknowledgement**: This work is part of the project supported by a grant No T 024145 from the Hungarian Scientific and Research Foundation (OTKA).*

REFERENCES

ARAI, T., SUGAWARA, M., SAKO, T., MOTOYOSHI, S., SHIMURA, T., TSUTSUI, N., KONNO, T. **(**1994**)** Glutathione peroxidase activity in tissues of chickens supplemented with dietary selenium, *Comp. Biochem. Physiol*. **107A**, 245-248

AVISSAR, N., EISENMANN, C., BREEN, J.G., HOROWITZ, S., MILLER, R.K., COHEN, H.J. (1994) Human placenta makes extracellular glutathione peroxidase and secretes it into maternal circulation, *Am. J. Physiol.* **267**: E68-76

BERSÉNYI, A., HULLÁR, I., FEKETE, S., HUSZENYICZA, GY., KÁDÁR, I., SZILÁGYI, M., GLÁVITS, R., MÉZES, M., KONCZ, J. (1998) Study the effect of carrot produced on soil containing high level of Se-, Mo and heavy metal (Cd, Pb, Hg) in a rabbit nutrition experiment In: *Role of selenium in the environment and health* Ed: Cser, M., Sziklainé lászló, I. FRAG bt. Budapest

BURK, R.F., BROWN, D.G., SEELY, R.J., SCAIEF, C.C.**(**1972**)** Influence of dietary and injected selenium on whole body retention, route of excretion, and tissue retention of $^{75}SeO_3^{2-}$ in the rat, *J. Nutr*., **102**: 1049

BURK, R.F. **(**1986**)** Selenium and cancer: meaning of serum selenium levels, *J. Nutr*. **116**: 1584-1586

CANTOR, A.H., TARINO, J.Z. (1982) Comparative effects of inorganic and organic dietary sources of selenium on selenium levels and selenium-dependent glutathione peroxidase activity in blood of young turkeys. *J. Nutr*. **112**:2187-2196

CLAUSEN, J., NIELSEN, S.A. **(**1988**)** Comparison of whole blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenate, selenit, L-selenomethioneine, and high selenium yeast, *Biol. Trace Elem. Res.* **15**: 125-138

GAÁL, T. **(**1998**)** Glutathione peroxidase activity and selenium status, In: Role of selenium in the environment and health Ed: Cser, m., Sziklainé lászló, I. FRAG bt. Budapest

HAFEMAN, D.G., SUNDE, R.A., HOEKSTRA, W.G. **(**1974**)** : Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat, *J. Nutr*. **104**: 580-587

HEIMANN, E.D., SMITH, M.F., MORRIS, J.S., GALL, T.J., ELMORE, R.G., MORROW, R.E. **(**1984**)** Relationships among spermatozoal abnormalities and the selenium concentration of blood plasma, semen and reproductive tissues in young bulls, *Anim. Repr. Sci*., **7**: 315-321

HUMALOJA, T., MYKKANEN, H.M. **(**1986**)** Intestinal absorption of ^{75}Se-labeled sodium selenite and selenomethioneine in chicks: effects of time, segment, selenium concentration and method of measurement, *J. Nutr.* **116**:142-148.

LEI,, X.G., DANN, H.M., ROSS, D.A., CHENG, W.H., COMBS, G.F., RONEKER, K.R. **(**1998**)** Dietary selenium supplementation is required to support full expression of three selenium-dependent glutathione peroxidases in various tissues of weanling pigs, *J. Nutr*. **128**: 130-135

LEIST, M., MAURER, S., SCHULTZ, M., ELSNER, A., GAWLIK, D., BRIGELIUS-FLOHÉ, R. **(**1999**)** Cytoprotection against lipid hydroperoxides correlates with increased glutathione peroxidase

activities, but not selenium uptake from different selenocompounds, *Biol. Trace Elem. Res*. **68**: 159-174.

LINGAAS, E., BRUN, E., FROSLIE, A. **(**1991**)** Estimates of heritability for selenium and glutathione peroxidase levels in pigs, *J. Anim. Breed. Genet*. **108**: 48-53

LOWRY, O.H., ROSENBROUGH, N. J., FARR, A. L., RANDALL, R.J. **(**1951**)** Protein measurement with Folin phenol reagent, *J. Biol. Chem.* **193**: 265-267

MAHAN, D.C., PARRETT, N.A. **(**1996**)** Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine, *J. Anim. Sci*. **74**: 2967-2974.

MATEOS, G., PIQUER, J. (1994) Disenno de programas alimentitios para conejos: aspectos teóricos y formulación práctica. *Boletin de Cunicultura* **17**:1631

MATKOVICS, B., SZABÓ, L., SZ. VARGA, I. **(**1988**)** *Laboratóriumi Diagnosztika* **48**: 248-250.

SANKARI, S., ATROSHI, F. **(**1983**)** Effect of dietary selenium on erythocyte glutathione peroxidase and blood selenium in two types of Finnsheep genetically selected for high and low glutathione peroxidase activity, *Zbl. Vet. Med*. **30**: 452-458.

SHEN, H.M., SHI, C.Y., LEE, H.P., ONG, C.N. (1994) Aflatoxin B1-induced lipid peroxidation in rat liver. *Toxicol. Appl. Pharmacol*. **127**: 145-150

SMITH, P.J., TAPPEL, A.L., CHOW, C.K. (1974**)** Glutathione peroxidase activity as a function of dietary selenomethionine, *Nature* **247**: 392

TOYODA, H., HIMENO, S., IMURA., N. **(**1989**)** The regulation of glutathione peroxidase gene expression relevant to species difference and the effects of dietary selenium manipulation, *Biochim. Biophys. Acta* **1008**: 301-308.

WHANGER, P., VENDELAND, S., PARK, Y.C., XIA, Y. **(**1996**)** Metabolism of subtoxic levels of selenium in animals and humans, *Ann. Clin. Lab. Sci*. **26**: 99-113

WOLFFRAM, S., ARDUSER, F., SCHARRER, E. **(**1985**)** In vivo intestinal absorption of selenate and selenite in rats, *J. Nutr*. **115**: 454

YOSHIDA, M., FUKUNAGA, K., TSUCHITA, H., YASUMOTO, K. **(**1999**)** An evaluation of the bioavailability of selenium in high-selenium yeast, *J. Nutr. Sci. Vitaminol (Tokyo)* **45**: 119-128