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Original article

The concentration of copper, zinc, manganese and selenium in the serum and liver of goats with caprine arthritis-encephalitis

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Abstract

Concentrations of four trace elements, copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se), have thus far proven to be affected by lentiviral infections in people and rhesus monkeys. As small ruminant lentivirus (SRLV) infection is responsible for one of the most important goat diseases, caprine arthritis-encephalitis (CAE), we evaluated serum and liver concentrations of Cu, Zn, Mn, Se in goats severely affected by symptomatic CAE and compared them with literature reference intervals. Serum and liver samples of dairy goats euthanized due to severe clinical form of CAE were collected and screened for the concentration of Cu, Zn, Mn (54 serum samples, 22 liver samples), and Se (36 serum samples, 22 liver samples) using flame atomic absorption spectrometry for Cu, Zn, Mn and graphite furnace atomic absorption spectroscopy for Se. In both serum and liver samples concentration of Zn was the highest, followed by Cu concentration, and then by Mn and Se. There was no relationship between serum and liver concentrations of trace elements. Liver concentrations of all four trace elements and serum Cu concentration fell within literature reference intervals, although liver Se concentration was mainly in the lower marginal range (between 0.4 and 1.0 mg/L). Serum Zn concentration was elevated (>1.2 mg/L) in all goats, serum Mn concentration was elevated (>0.04 mg/L) in 42 (78%) goats and serum Se concentration was elevated (>1.6 mg/L) in 13 (36%) goats. Concluding, severe symptomatic CAE does not appear to be associated with the level of any of the four trace elements.

Key words: caprine arthritis-encephalitis, copper, goats, lentivirus, manganese, selenium, zinc

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Introduction

Trace elements (microminerals) are mineral micronutrients essential for the proper function of animal organisms. They include cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn). Being constituents of many compound proteins such as metalloenzymes they perform many catalytic and regulatory functions (Suttle 1996). Their levels in the organism depend not only on the dietary intake but also on the rate of absorption from the alimentary tract and the capability of retaining them in the tissues. Therefore, their levels may be affected by chronic diseases. This phenomenon has been described in people with chronic heart failure (von Haehling et al. 2007, Krim et al. 2013), chronic kidney disease (Zachara et al. 2006), various neoplastic diseases (Ströhle et al. 2010), and acquired immunodeficiency syndrome (AIDS). Patients with AIDS have been shown to have reduced blood levels of Mn (Afridi et al. 2014) and Zn (Baum et al. 2003), and increased Cu levels (Beck et al. 1990, Bilbis et al. 2010, Afridi et al. 2011). However, the strongest relationship has been established between the reduced Se level and lentiviral infection in people (i.e. human immunodeficiency virus, HIV) (Allard et al. 1998, Di Bella et al. 2010) and Rhesus monkeys (i.e. simian immunodeficiency virus, SIV) (Xu et al. 2002). Furthermore, lower Se levels have proven to be causally linked to the progression of HIV infection into symptomatic form, likely due to impairment of the antioxidant defense status (Constans et al. 1995, Baum et al. 1997, Look et al. 1997).

Caprine arthritis-encephalitis (CAE) is caused by small ruminant lentivirus (SRLV) infection and bears many similarities to HIV and SIV infection (Thormar 2005). After long subclinical development it results in chronic progressive arthritis and ill thrift, potentially leading to cachexia (Smith and Sherman 2009). Since concentration of trace elements in the course of CAE has never been investigated, we determined serum and liver concentrations of four trace elements, known to be affected by lentiviral infection in people (Cu, Zn, Mn, Se), in goats severely affected by symptomatic CAE and compared them with literature reference intervals.

Materials and Methods

The study was carried out in the spring 2017 in a large dairy goat herd (roughly 400 adult female goats) from the northern Poland. Goats in the herd were kept in a loose-housed concrete barn. They were not grazed on pasture but fed hay, haylage, corn silage and roughly 1 kg of protein concentrates consisting mainly of barley (30%), brewer's threshing (30%), rapeseed meal (20%), wheat bran (15%), and soybean meal (5%). They had free access to mineral blocks (Solsel, Poland) containing Mn (1 g/kg), Zn (1 g/kg), J (0.1 g/kg), Se (0.02 g/kg), and Fe (0.2 g/kg). The herd had been affected by SRLV infection for at least 20 former years and two serological surveys carried out in 2014 and 2016 showed that the true within-herd seroprevalence tended to approach 100%.

Fifty four goats of Polish White Improved and Polish Fawn Improved breed aged from 1.8 to 11.8 years with the median of 5.7 years (interquartile range from 3.7 to 7.0 years) were enrolled in the study. They were intended for sanitary culling due to severe clinical form of CAE. They all had tested seropositive in ELISA (ID Screen MVV/CAEV Indirect-Screening test) in the serological survey from 2016. They were clinically examined by two board-certified specialists in small ruminant health management (JK and MC) and were shown to manifest severe lameness, bilateral swelling of carpal joints and emaciation with lumbar and sternal scores of 0 or 1 (Matthews 2009). Blood samples were collected from the jugular vein via the indwelling intravenous catheter (16G) placed to inject general anesthetics. Then, the animals were anesthetized with the intravenous mixture of ketamine 5 mg/kg (Vetaketam 10%, Vet-Agro, Poland) and xylazine 0.1 mg/kg (Xylapan 2%, Vetoquinol, Poland), and euthanized by intravenous overdosing of pentobarbital 30 mg/kg (Morbital 16%, Biowet Puławy, Poland). The autopsy was performed within 2 hours. At autopsy the liver was visually inspected and the fragment of the left lateral lobe weighing roughly 100g was collected from 22 goats (41% of all 54 goats) and immediately frozen at -20°C until testing. Blood was left overnight in refrigerator (2-8°C), then centrifuged at 3000 × g for 10 min. and frozen at -20°C in 2ml vials until testing. Concentrations of Cu, Zn and Mn were measured in all 54 serum samples, while Se concentration in 36 serum samples.

The liver samples (n=22) were dried to constant weight at 70°C. About 1g of serum and liver were digested by applying nitric acid 65% (v/v) and hydrogen peroxide solution 30% (v/v) in H_2O at 100±20°C with block digester (VELP). Concentrations of Cu, Zn and Mn were determined by the flame atomic absorption spectrometry (F-AAS), and concentration of Se by the graphite furnace atomic absorption spectroscopy (GF-AAS) using a spectrometer M6 Solar Thermo Elemental (Thermo Scientific, Wilmington, DE).

Numerical variables were presented as the median, interquartile range (IQR) and range, and they were compared between groups using the Mann-Whitney U

Table 1. Concentration of copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) in the serum and liver samples of goats affected by severe symptomatic caprine arthritis-encephalitis (given as the median, interquartile range (IQR) and the range) along with literature reference intervals.

Trace element	Serum		Liver		Reference intervals	
	n	mg/L	n	mg/kg dry weight (ppm)	Serum (mg/L)	Liver (mg/kg)
Cu[a]	54	1.28, 1.08-1.47 (0.67-1.96)	22	51.2, 22.6-81.2 (12.6-277.8)	0.6 to 1.5-2.0 (Suttle 1996)	10 to 250-300 (Puls 1994, Suttle 1996)
Zn[b]	54	1.90, 1.60-2.25 (1.13-4.78)	22	105.9, 87.5-123.4 (68.0-138.8)	0.6 to 1.2 (Suttle 1996)	20 to 120 (Puls 1994)
Mn[c]	54	0.05, 0.03-0.09 (<0.01-0.53)	22	5.75, 4.77-7.12 (3.38-9.21)	<0.04 (Hidiroglou 1979)	3 to 6 (Suttle 1996)
Se[d]	36	0.13, 0.09-0.29 (0.03-0.89)	22	0.58, 0.50-0.73 (0.32-1.37)	0.06 to 0.16 (Smith and Sherman 2009)	0.4-1.0 to 4.8 (Van Metre and Callan 2001)

[a] multiply by 15.74 to convert mg/L (mg/kg dry weight) to µmol/L (µmol/kg dry weight)
[b] multiply by 15.29 to convert mg/L (mg/kg dry weight) to µmol/L (µmol/kg dry weight)
[c] multiply by 18.20 to convert mg/L (mg/kg dry weight) to µmol/L (µmol/kg dry weight)
[d] multiply by 12.66 to convert mg/L (mg/kg dry weight) to µmol/L (µmol/kg dry weight)

test. Concentrations of trace elements were compared separately in the serum and liver samples using repeated measure ANOVA with Tukey HSD post-hoc test performed on rank-transformed data (Conover and Iman 1981). The relationship between serum and liver concentration of minerals was determined using the Spearman's rank-order correlation coefficient (r_s). A significance level (α) was set at 0.05. Statistical analysis and plots were prepared in Statistica 12 (StatSoft, Inc., USA).

Results

Age of 54 goats enrolled in the study did not differ between goats whose liver samples were (n=22) or were not (n=32) tested (p=0.805).

Concentrations of trace elements differed significantly both in serum (p<0.001) and liver samples (p<0.001) (Table 1). In both serum and liver samples concentration of Zn was the highest, followed by Cu concentration (p<0.001), and then by Mn and Se which were present at the lowest concentrations (p<0.001). However, in the serum samples Se concentration was significantly higher than Mn concentration (p<0.001) (Fig. 1), while in the liver samples the ratio was opposite (p<0.001) (Fig. 2).

There was no relationship between serum and liver concentrations of trace elements:

Cu – r_s = 0.18 (p=0.414);
Zn – r_s = -0.02 (p=0.922),
Mn – r_s = -0.25 (p=0.257),
and Se – r_s = 0.19 (p=0.471).

Liver concentrations of all four trace elements as well as serum Cu concentration fell within commonly accepted reference intervals, although liver Se concentration was mainly in the lower marginal range (between 0.4 and 1.0 mg/L). On the other hand, serum Zn concentration was above the upper limit of the reference interval (1.2 mg/L) in all tested goats, serum Mn concentration was increased (above 0.04 mg/L) in 42 (78%) goats and serum Se concentration was increased (above 1.6 mg/L) in 13 (36%) goats.

Discussion

Our study did not reveal any substantial abnormalities in the concentration of four trace elements in goats with severe symptoms of CAE. Obviously, an important drawback to this study is the lack of a control group. As it was a case series, not an observational study, we could not compare concentration of track elements either between SRLV-infected and non-infected goats or between SRLV-infected goats with and without severe clinical signs. For our excuse we may say that enrolling a seronegative control group in the herd like ours would have been almost impossible – even if we had found enough seronegative goats, their true health status would have still been dubious due to very low negative predictive value of the serological test result in the herd with widespread infection (Nowicka et al. 2014).

Therefore, the only analysis we could perform was a comparison with literature reference intervals. However, reference intervals depend on the manner in which they have been determined such as laboratory

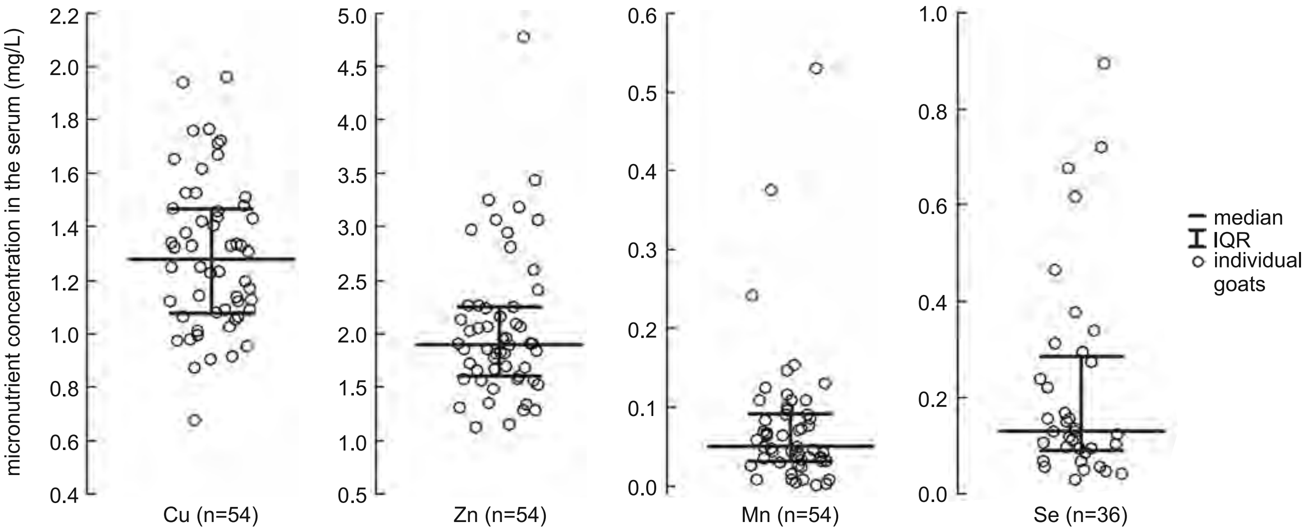

Fig. 1. Concentration of copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) in the serum samples of goats affected by severe symptomatic caprine arthritis-encephalitis.

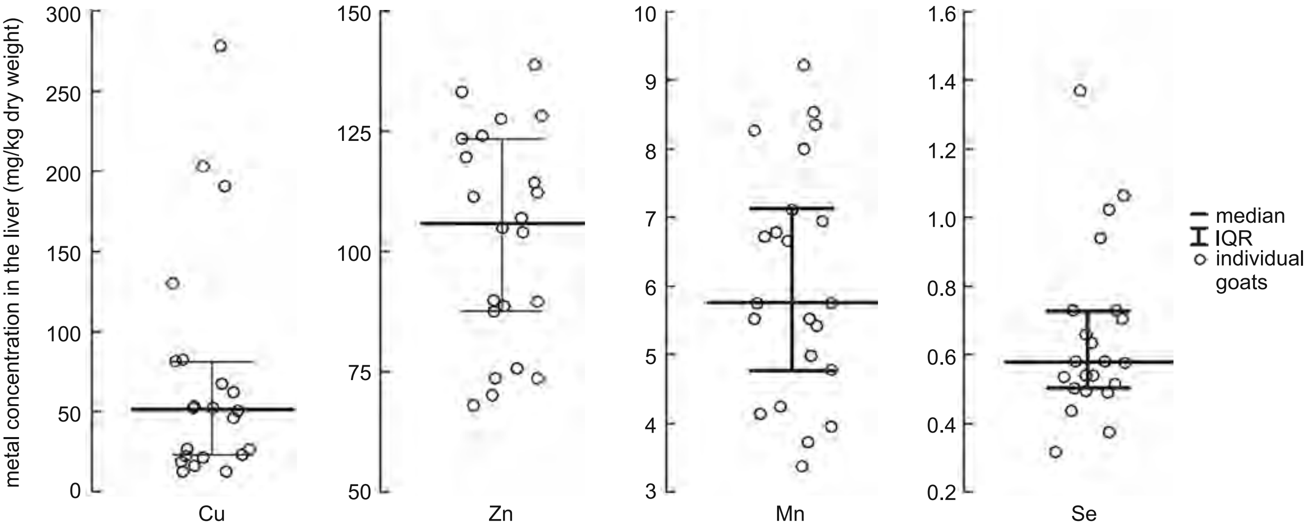

Fig. 2. Concentration of copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) in the liver samples of 22 goats affected by severe symptomatic caprine arthritis-encephalitis.

and epidemiological methodology used as well as characteristics and size of a target population enrolled. Therefore, it is recommended that each laboratory develop its own reference intervals (Friedrichs et al. 2012). Using reference intervals obtained from the literature, without taking into account the specific setting in which a given study has been conducted, bears considerable risk of drawing false conclusions. On the other hand, reference intervals are by principle determined on the basis of clinically healthy individuals. Therefore, having a given parameter outside the reference interval does not automatically classify an individual as affected by the disease diagnosed on the basis of this parameter alteration (indeed mild deviations from a reference interval are infrequently of clinical value). However, being in the middle of a reference interval is quite a strong evidence of belonging to the healthy population.

In our study serum concentrations of Zn, Se and Mn were higher than expected according to literature reference intervals. This observation implies that SRLV infection is unlikely to be associated with impaired absorption of trace elements contrary to what has been observed in HIV-infected people (Baum 2000). Furthermore, some trace elements, especially Se and Zn have been shown to influence the prognosis in HIV infected patients – Se-deficient patients are more likely to progress to further stages of disease (Cirelli et al. 1991), develop both co-infections (Shor-Posner et al. 2002) and non-infectious complications (Twagirumukiza et al. 2007), and eventually to die sooner (Baum et al. 1997). This is probably associated with an important role of those trace elements in immunologic and antioxidant defense mechanisms. However, SRLV-infection elicits chronic immune-mediated inflammatory processes, mainly in joints (Stonos et al. 2014) and is not considered to cause immunosuppression (Thormar 2005). Hence, deficit of natural immune-stimulating factors like Se or Zn does not have to be as crucial in the progression from asymptomatic to arthritic form of CAE as it is in the development of AIDS. High levels of Zn, Se and Mn observed in our study probably resulted from efficient mineral supplementation practiced in this herd. Mineral blocks were placed all over the barn so that goats could access them instantly, and according to our personal observations, even goats severely affected by CAE were eager to do this many times during the day. Moreover, mineral blocks contained all trace elements we measured except for Cu and this was the only trace element that remained halfway the reference interval.

Interestingly, high serum Se levels were accompanied by low liver Se concentrations. This may indicate impairment of Se liver storage in CAE-affected animals, however we do not know any molecular mechanism potentially accounting for this phenomenon, neither have we found any other publication substantiating this observation. Thus, it undoubtedly needs verification in the observational study conducted with a control group.

In conclusion, severe symptomatic CAE does not appear to be associated with the deficiency of any of four trace elements, which have so far proven to be affected by the lentiviral infection in people.

References

Afridi HI, Kazi TG, Kazi N, Kandhro GA, Baig JA, Shah AQ, Khan S, Kolachi NF, Wadhwa SK, Shah F (**2011**) Evaluation of zinc, copper and iron in biological samples (scalp hair, blood and urine) of tuberculosis and diarrhea male human immunodeficiency virus patients. Clin Lab 57: 677-688.

Afridi HI, Kazi TG, Talpur FN, Arain S, Arain SS, Kazi N, Panhwar AH, Brahman KD (**2014**) Evaluation of chromium and manganese in biological samples (scalp hair, blood and urine) of tuberculosis and diarrhea male human immunodeficiency virus patients. Clin Lab 60: 1333-1341.

Allard JP, Aghdassi E, Chau J, Salit I, Walmsley S (**1998**) Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am J Clin Nutr 67: 143-147.

Baum MK, Campa A, Lai S, Lai H, Page JB (**2003**) Zinc status in human immunodeficiency virus type 1 infection and illicit drug use. Clin Infect Dis 37 Suppl 2: S117-123.

Baum MK (**2000**) Role of micronutrients in HIV-infected intravenous drug users. J Acquir Immune Defic Syndr 25: S49-S52.

Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB (**1997**) High risk of HIV-related mortality is associated with selenium deficiency. J Acquir Immune Defic Syndr Hum Retrovirol 15: 370-374.

Beck KW, Schramel P, Hedl A, Jaeger H, Kaboth W (**1990**) Serum trace element levels in HIV-infected subjects. Biol Trace Elem Res 25: 89-96.

Bilbis LS, Idowu DB, Saidu Y, Lawal M, Njoku CH (**2010**) Serum levels of antioxidant vitamins and mineral elements of human immunodeficiency virus positive subjects in Sokoto, Nigeria. Ann Afr Med 9: 235-239.

Cirelli A, Ciardi M, de Simone C, Sorice F, Giordano R, Ciaralli L, Costantini S (**1991**) Serum selenium concentration and disease progress in patients with HIV infection. Clin Biochem 24: 211-214.

Conover WJ, Iman RL (**1981**) Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 35: 124-129.

Constans J, Pellegrin JL, Sergeant C, Simonoff M, Pellegrin I, Fleury H, Leng B, Conri C (**1995**) Serum selenium predicts outcome in HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 10: 392.

Di Bella S, Grilli E, Cataldo MA, Petrosillo N (**2010**) Selenium deficiency and HIV infection. Infect Dis Rep 2: e18.

Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J (**2012**) ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol 41: 441-453.

Hidiroglou M (**1979**) Manganese in ruminant nutrition. Can J Anim Sci 59: 217-236.

Krim SR, Campbell P, Lavie CJ, Ventura H (**2013**) Micronutrients in chronic heart failure. Curr Heart Fail Rep 10: 46-53.

Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, Sudhop T, Hoch J, Stockinger K, Spengler U, Sauerbruch T (**1997**) Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection. Eur J Clin Nutr 51: 266-272.

Matthews JG (**2009**) Diseases of the Goat. 3rd ed., Wiley-Blackwell, Ames, Iowa, USA, pp 124-126.

Nowicka D, Czopowicz M, Mickiewicz M, Szaluś-Jordanow O, Witkowski L, Bagnicka E, Kaba J (**2014**) Diagnostic performance of ID screen MVV-CAEV Indirect Screening ELISA in identifying small ruminant lentiviruses-infected goats. Pol J Vet Sci 17: 501-506.

Puls R (**1994**) Mineral levels in animal health. Diagnostic data. 2nd ed., Sherpa International, Clearbrook, Canada, pp 100-101.

Shor-Posner G, Miguez MJ, Pineda LM, Rodriguez A, Ruiz P, Castillo G, Burbano X, Lecusay R, Baum M (**2002**) Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 29: 169-173.

Smith MC, Sherman DM (**2009**) Goat medicine. 2nd ed., Wiley-Blackwell, Ames, Iowa, USA, pp 96-106.

Stonos N, Wootton SK, Karrow N (**2014**) Immunogenetics of small ruminant lentiviral infections. Viruses 6: 3311-3333.

Ströhle A, Zänker K, Hahn A (**2010**) Nutrition in oncology: the case of micronutrients (review). Oncol Rep 24: 815-828.

Suttle NF (**1996**) Mineral nutrition of livestock. 4th ed., CAB International, Cambridge, USA, pp 1-4.

Thormar H (**2005**) Maedi-visna virus and its relationship to human immunodeficiency virus. AIDS Rev 7: 233-245.

Twagirumukiza M, Nkeramihigo E, Seminega B, Gasakure E, Boccara F, Barbaro G (**2007**) Prevalence of dilated cardiomyopathy in HIV-infected African patients not receiving HAART: a multicenter, observational, prospective, cohort study in Rwanda. Curr HIV Res 5: 129-137.

Van Metre DC, Callan RJ (**2001**) Selenium and vitamin E. Vet Clin North Am Food Anim Pract 17: 373-402.

von Haehling S, Doehner W, Anker SD (**2007**) Nutrition, metabolism, and the complex pathophysiology of cachexia in chronic heart failure. Cardiovasc Res 73: 298-309.

Xu XM, Carlson BA, Grimm TA, Kutza J, Berry MJ, Arreola R, Fields KH, Shanmugam I, Jeang KT, Oroszlan S, Combs GF Jr, Marx PA, Gladyshev VN, Clouse KA, Hatfield DL (**2002**) Rhesus monkey simian immunodeficiency virus infection as a model for assessing the role of selenium in AIDS. J Acquir Immune Defic Syndr 31: 453-463.

Zachara BA, Gromadzińska J, Wasowicz W, Zbróg Z (**2006**) Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: a review. Acta Biochim Pol 53: 663-677.