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Effects of commercial selenium products on glutathione peroxidase activity and semen quality in stud boars

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ABSTRACT

The aim of this study was to determine how dietary supplementation of inorganic and organic selenium affects the selenium concentration and glutathione peroxidase activity in blood and sperm of sexually mature stud boars. Twenty-four boars of the Large White, Landrace, Pietrain and Duroc breeds of optimal breeding age (on average 2.5 years old) were used. The study lasted 90 days. The boars were randomly assigned to one of three dietary treatment groups: T1 = control; no added selenium ($n = 8$ boars), T2 = added 0.3 ppm inorganic selenium (sodium selenite, Microgran® Se 1% BMP) ($n = 8$ boars), and T3 = added 0.3 ppm organic selenium (Se-yeast, Sel-Plex 2000®) ($n = 8$ boars). The concentration of selenium was determined in whole blood and semen, while the activity of glutathione peroxidase (GPx) was measured in blood plasma and semen. In order to measure GPx activity in semen, reactivation of the enzymatic GPx activity was performed. The determined selenium concentration in blood was lowest in the non-supplemented group of boars. Blood plasma GPx activity was higher in boars fed organic selenium than in boars fed a diet without supplemented selenium. While the supplementation of sodium selenite significantly increased GPx activity in boar semen. The highest-concentration of selenium in semen at the end of the trial was determined in the group of boars supplemented with organic selenium, somewhat lower in boars fed supplemented inorganic selenium, and the lowest in the non-supplemented group of boars. The only significant difference between the selenite and Se-yeast diet supplementation was observed in the Se concentration of the semen. The supplementation of selenium affected semen quality, and organic selenium improved the progressive motility of the spermatozoa and increased their resistance in hypo-osmotic and thermal tests. The storage ability of short term preserved semen was improved by organic selenium supplementation, as well as also increasing the fertility rate in gilts.

Abbreviations: AI, artificial insemination; β-NADPH, β-nicotinamide adenine dinucleotide 2′-phosphate; GPx, glutathione peroxidase; LMNS, live morphologically normal spermatozoa; NRC, National Research Council; PHGPx, phospholipid hydroperoxide glutathione peroxidase; PUFA, polyunsaturated fatty acids; TBH, *t*-butyl hydroxide; TGR, thioredoxin/glutathione reductase; $^{P}H_2O$, type 2 pure water; XFM, X-ray fluorescence microscopy.

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1. Introduction

Selenium is an essential trace element necessary for reproduction and embryonic growth. Selenium is known to be required for testosterone biosynthesis as well as for formation and development of spermatozoa (Behne et al., 1996). It has been shown that the mammalian selenium protein thioredoxin/glutathione reductase (TGR) has a role in disulfide bridge formation during the sperm maturation process (Su et al., 2005).

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) or GPx4 is the fourth described selenoprotein from the family of glutathione peroxidases (GPxs) (Ursini et al., 1982). GPx4 is specific due to the fact that beside glutathione it can use a wide array of reducing substrates (Aumann et al., 1997) and is mostly present in testicles (Imai et al., 1995). The primary structural difference between GPxs is that GPx4 is a protein monomer while all other GPxs are tetramers. Since GPx4 can exist close to the membrane, it has important consequences for membrane protection (*in situ*) (Ursini et al., 1982). Recently, three different isoforms of GPx4: mitochondrial, non-mitochondrial and sperm nuclei specific forms have been characterized (Schneider et al., 2009).

Alvarez and Storey (1989) were the first to describe the role played by GPx in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. Many years later, it was reported that failure of GPx expression in spermatozoa was correlated with infertility in humans (Imai et al., 2001; Foresta et al., 2002). Within the last 6 years, the development of mouse GPx knockout models (Schneider et al., 2009; Imai et al., 2009; Liang et al., 2009) resulting in infertility or subfertility have demonstrated that GPx does indeed play an important role in mammalian sperm physiology.

Since the role that GPx plays in the removal of free radicals is well known, it is then not suprising that GPx is important in the protection of spermatozoa during spermatogenesis. The explaination for this is clear since lipid peroxidation was significantly increased either by GPx inhibition with mercaptosuccinate, a specific Se dependant GPx inhibitor, or by a decrease in the level of available reduced glutathione—GSH (Alvarez and Storey, 1989). The role of GPx in the protection of spermatozoa and male germinative tissue from peroxidative damage is even more important since catalase in mammal spermatozoa or sperm plasma is not present in significant amounts (Alvarez et al., 1987; Bilodeau et al., 2000). Therefore, in the absence of catalase, GPx plays a crucial role in the protection of spermatozoa during development and of testicular tissue from the effects of hydrogen peroxide. The GPx in spermatids (the developmental stage of spermatozoa) is in the form of the active peroxidase, while in mature spermatozoa it is transformed into structural protein (inactivated enzyme) which is incorporated in the mitochondrial capsule of spermatozoa. The mitochondrial capsule and the kinetic apparatus are located in the middle section of spermatozoa (Ursini et al., 1999). Alternatively to catalase, which reacts only with H_2O_2 as substrate and in relatively high-concentrations ($>10^{-6}$ M), GPx allows the subtle regulation of the H_2O_2 concentration. GPx also enables the repair of complex molecules (an example is cell membrane lipids) which are damaged by H_2O_2.

The spermatozoal plasma membrane is rich in PUFA (polyunsaturated fatty acids), the target of free radicals, which additionally emphasizes the significance of the protection afforded by GPx, as well as the fact that selenium is a necessary component of GPx, which makes the trace element selenium of utmost importance in reproduction. Also, widespread selenium deficiency can lead to lower egg production and decreased reproduction efficiency (Surai, 2002).

Mihailović et al. (1996) and Jovanović et al. (1998) have analyzed the selenium content in a total of 276 feed samples collected from 55 localities throughout Serbia and found the average selenium concentration to be 30.4 ± 25.3 μg/kg, which ranges from marginal deficiency (49.5 ± 25.3 μg/kg) in the northern part of the country to severe deficiency (8.8 ± 5.7 μg/kg) in the Sjenica-Pešter plateau in the Southwest region. Dimitrov et al. (2007) reported the beneficial effects of selenium supplementation on turkey semen. It is generally considered that organic forms of supplemented selenium have higher-biological availability when compared with inorganic forms in most domestic animals (Daniels, 1996), including swine (Mahan and Peters, 2004; Fortier and Matte, 2006).

The aim of this study was to determine how the supplementation of dietary inorganic and organic selenium affects the selenium concentration, GPx activity in blood and sperm as well as boar semen quality and fertility in sexually mature stud boars.

2. Materials and methods

2.1. Materials

Inorganic selenium (sodium selenite) Microgran® Se 1% BMP, was obtained from DSM Nutritional Products Ltd., Basel, Switzerland. Organic selenium (Se-yeast) Sel-Plex 2000® was obtained from Alltech Inc., Dublin, Ireland. HCl, 320331; HNO_3, 02650 (Fluka); H_2O_2, H0904; TraceCERT®, 1000 mg/L Pd in hydrochloric acid, 78437; glutathione reduced, G4251; glutathione reductase from baker's yeast, G3664; β-NADPH, N7505; β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate, 82059 (Fluka); *t*-butyl hydroxide, 58448; and 0.1 M $NaHCO_3$ in H_2O, 36486 were obtained from Sigma–Aldrich, St. Louis, MO, USA. Vacutainers, LH102 I.U., were purchased from BD, Plymouth, Great Britain; semen extender Androstar Plus® was purchased from Minitüb, Tiefenbach, Germany; and the disposable catheters, Golden Gilt® were purchased from Kruuse, Denmark.