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# Effect of Organic and Inorganic Form of Selenium on Antioxidant Status of Breeding Boars Ejaculate Revealed by Electrochemistry

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The main aim of the presented study was to determine the effects of selenium on antioxidant status of Duroc boars ejaculates. Twenty-eight Duroc boars were divided to four experimental groups, seven boars per each group. Each group was supplemented with both the organic form (0.3 and 0.6 mg selenium per kg) and inorganic form (0.3 and 0.6 mg selenium per kg) of selenium in the feed mixture. The duration of the experiment was 18 weeks. All the boars were carefully collected at strictly defined four time intervals. The activity of glutathione peroxidase, glutathione reductase, antioxidant capacity, ratio between reduced and oxidized glutathione, metallothionein content and concentration of zinc and selenium were determined. The dietary supplementation of the organic form of selenium at a dose of 0.6 mg/kg of feed mixture increased significantly (by 48 %, $P < 0.01$) activity of the glutathione peroxidase in the ejaculate. Concentration of selenium in ejaculate of this experimental group was increased by 82 % ($P < 0.05$). Dietary supplementation of organic form of selenium at a dose of 0.6 mg/kg of feed mixture led to an increase of free radicals by 62 % ($P < 0.01$), ABTS for 45 % ($P < 0.05$), and GSH/GSSH for 55 % ($P < 0.05$). When evaluating the content of zinc and metallothionein, no significant differences between experimental groups of animals were found. Experimental group of boars supplemented by the selenium in the concentration of 0.3 mg/kg in the feed mixture in the inorganic form had significantly reduced the total number of sperms produced per one taking ($P <0.001$). The results of the present study indicate that the dietary supplementation of feed mixture by the selenium in the concentration of 0.6 mg Se/kg significantly improves the antioxidant potential of the breeding boars ejaculate. A lower oxidative stress and higher fertility can be therefore assumed.

**Keywords:** selenomethionine; sodium selenite; antioxidant status; zinc; metallothionei boars; differential pulse voltammetry; high performance liquid chromatography with electrochemical detection

*LIST OF THE ABBREVIATIONS*
ABTS - 2,2-azinobis(3-ethylbenzothiazolin-6-sulphonic acid); DPPH - 2,2-diphenyl-1-picrylhydrazyl - DPPH•; FM – feed mixture; FR – free radicals; GSH-Px- glutathione peroxidase; GR – glutathione reductase; GSH/GSSG – reduced and oxidized glutathione; G1- the first experimental group of boars (0.3 mg Se/kg feed mixture in the organic form); G2 - the second experimental group of boars (0.3 mg Se/kg feed mixture in the inorganic form); G3 - the third experimental group of boars (0.6 mg Se/kg feed mixture in the organic form); G4 - the fourth experimental group of boars (0.6 mg Se/kg feed mixture in the inorganic form); ISB – insemination station boars; MT – metallothionein; ROS – reactive oxygen species; Se – selenium; Zn – zinc

## 1. INTRODUCTION

Selenium is an essential element for reproduction of boars [1], in spite of the fact that selenium has been considered as a substance causing toxicity in livestock for many years [2]. Selenium plays an important role in the correct sequence of physiological functions, especially in farm animals [3]. Selenium is ubiquitous [4]. Selenium is a component of the enzyme glutathione peroxidase, which is one of the most important antioxidants in animals and marker of oxidative stress [5,6]. Imbalance between reactive oxygen species and total antioxidant capacity can cause male infertility [7,8]. Selenium as a component of glutathione peroxidase protects spermatozoa, spermatogonia and sperm cells themselves against the free oxygen radicals [9]. In addition, it plays crucial role during the process of spermatogenesis [10]. Therefore, it is not surprising that selenium is essential for the sperm cells, because it provides their viability, motility and generally total fertility of males [11,12]. Selenium itself influences not only the quality of ejaculate of boars, but participates in the reproduction processes of gilts [13,14]. Selenium deficiency in the diet leads to the decline of ejaculate quality, which causes deteriorated fertilizing ability [15]. Two selenium forms, organic and inorganic, are available, whereas it is assumed that organic form is bioavailable and has reduced toxicity compared to inorganic one [16,17]. The recommended dose of selenium is 0.3 mg/kg in feed mixture for modern genotypes of breeding boars [18]. Supplementation of diet of pigs with selenium may be very useful. In this case, so-called functional food can be obtained. These are considered to be beneficial to human health [19]. In conclusion, selenium may be used as an essential trace element in the nutrition of animals and people [20,21]. In addition, zinc plays important role in the production of testosterone and thus influences the male fertility [22]. Zinc is, together with selenium, important antioxidant [23].

In spite of the well known doses of selenium, pathways involved in selenium metabolism are not fully understood. There should be involved numerous proteins including metallothioneins (MT). These protein were discovered by Margoshes and Valee in 1957 as newly identified proteins isolated from a horse renal cortex tissue [24]. They occur in whole animal kingdom with high degree of homology. Similar proteins are expressed by bacteria, fungi and even plants. MTs are low molecular mass (from 2 to 16 kDa) proteins with unique abundance of cysteine residues (more than 30 % from all aminoacids). Other interesting structural property is the lack of aromatic amino acids. The main

function of MTs in organism is a metal ion transport, maintenance of the oxidative-reducing conditions and regulation of gene expression. Thiols including MT and glutathione are known as effective radical scavengers creating optimal oxidative-reducing conditions; as a result, cell compartments and biologically important compounds including cell-cycle-enzymes or DNA are protected [25,26]. MT regulates free radical level also indirectly by binding of metal ions which are potential radical producers, e.g. Cu [27,28]. As confirmed by several studies [29,30], MT expression in cells is induced also by superoxide and hydroxyl radicals generated by γ-radiation. It is supposed that MT acts either as a scavenger of radicals or zinc donor for enzymes participating in repairing processes [31]. Therefore, the main aim of this study was to compare the different levels and forms of selenium and their effect on the antioxidant status of breeding boars ejaculate.

## 2. EXPERIMENTAL PART

### *2.1 Experimental design*

The experiment was conducted at the Insemination Station for Boars (ISB) in Velké Meziříčí (Czech Republic). Twenty-eight Duroc boars were selected for this study and were divided into four well-balanced groups (according to the age). The age interval of boars ranged from 1 to 3 years. The experimental animals were housed individually and had free access to water. The first group (G1) boars (n = 7) was fed with 0.3 mg Se/kg of feed mixture (FM) in organic form. The second group (G2) boars (n = 7) was fed with 0.3 mg Se/kg of FM in inorganic form. The third group (G3) boars (n = 7) was fed with 0.6 mg Se/kg of FM in organic form.

**Table 1.** Composition of feed mixture for boars.

| Component | % representation |
|---|---|
| Barley grain | 36.00 |
| Wheat grain | 20.36 |
| Oat grain | 20.00 |
| Soybean extracted scrap | 14.50 |
| EKPO T | 3.00 |
| Bergafat | 2.10 |
| Calcium carbonate | 1.50 |
| Monodicalcium phosphate | 1.20 |
| Mineral vitamin premix | 0.50 |
| Sodium chloride | 0.40 |
| Magnesium oxide | 0.15 |
| L-Lysine HCl | 0.14 |
| L-Threoninine | 0.09 |
| DL-Methionine | 0.06 |

Bergafat (Berg + Schmidt, Germany) – palm oil; EKPOT (Delika-Pet, Czech Republic) – biscuit meal

The last one (G4) boars (n = 7) was fed with 0.6 mg Se/kg of FM in inorganic form. As a source of inorganic selenium, sodium selenite was used. Selenium-enriched yeast was used for the selenium supplementation in organic form (Sel-Plex - Alltech's, USA). The composition of FM, which was the same for all experimental groups, is shown in Tab. 1.

The duration of the experiment was 18 weeks. The experimental period was started in the mid-April 2011 and ended in the mid-August 2011. All boars were trained for ejaculate collection (every third sampling) during this period. Ejaculate was collected at a regular frequency of every six (four) weeks. The first control sampling was performed before the experiment has begun. Native ejaculate was frozen immediately after the collection. Collection of ejaculate was performed manually using the phantom.

### *2.2 Determination of sperm cells concentration*

The concentration of sperm cells determined photometrically (Spekol 11, Carl Zeiss, Jena, UK) at 600 nm was expressed as the number of sperm per 1 $mm^3$ of an ejaculate. Calibration curve was used to calculate concentration of sperm cells.

### *2.3 Sample preparation for analyses*

Firstly, 0.5 ml volume of native thawed ejaculate was pipetted with subsequent addition of 2 ml of liquid nitrogen and 0.5 ml of phosphate buffer. Subsequently, the sample was homogenized in ULTRA-TURRAX T8 homogenizer (IKA, Konigswinter, Germany) at 3000 rpm for 2 minutes. After homogenization, 1 ml of phosphate buffer was added. Sample modified like this was homogenized in vortex (Vortex-2 Genie Scientific Industries, New York, NY, USA) at 2000 rpm for 15 minutes. Subsequently, the sample was centrifuged in Universal 32 R centrifuge (Hettich-Zentrifugen GmbH, Tuttlingen, Germany) at 16000 rpm at 4 °C for 20 minutes. Finally, supernatant was removed and used for analyses (1.5 ml).

### *2.4 Determination of glutathione peroxidase*

A Glutathione Peroxidase Cellular Activity Assay Kit (CGP1, Sigma Aldrich, USA) was used for the GPx assay, determination was done in ejaculates. For the determination of glutathione peroxidase activity, BS 400 automated spectrophotometer (Mindray, China) was used. The experimental protocol was as it follows. A 260 µl volume of reagent R1 (0.3 mM NADPH in GPx buffer) was pipetted into a plastic cuvette with subsequent addition of 10 µl of sample and after mixing, a 30 µl volume of reagent R2 (3 mM tert-butyl hydroperoxide) was added to the cuvette which starts the reaction. The decrease in absorbance was measured at 340 nm using kinetic program for 126 seconds.

*2.5 Determination of glutathione reductase*

A Glutathione Reductase Assay Kit (Sigma Aldrich, USA) was used for glutathione reductase (GR) activity,, determination was done in ejaculate. For determination of glutathione reductase activity, BS 400 automated spectrophotometer (Mindray, China) was used. Reagents R1 and R2 were prepared by dissolving in an assay buffer (100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA). A 260 µl volume of the reagent R1 (1.15 mM oxidized glutathione in the assay buffer) was mixed with 10 µl of sample and 30 µl volume of reagent R2 (1 mM NADPH in GR assay buffer) in a plastic cuvette. The decrease in absorbance was measured at 340 nm using a kinetic program for 126 s.

*2.6 Determination of antioxidant activity*

Solutions and ABTS, DPPH and FR assay were prepared according to Sochor et al. [32]. Brief description of the assays is below.

*2.6.1 Determination of antioxidant capacity using the ABTS test*

*Reagent preparation*: Seven mmol/l 2,2‘-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, m = 0.03841 g/10 ml) and 4.95 $mmol \cdot l^{-1}$ potassium peroxodisulphate (m = 0.01338 g/10 ml) are mixed and dissolved in ACS water. The solution is then diluted with ACS water in a 1:9 *v*/*v* ratio (10 ml is quantitatively transferred into 100 ml calibrated flask and diluted).

A 150 µl volume of the reagent was mixed with 3 µl of sample. Absorbance was measured at 660 nm. For calculating of the antioxidant activity, difference between absorbance at the last ($12^{th}$) minute and at the second minute of the assay procedure was used.

*2.6.2 Determination of antioxidant capacity using the DPPH$^{\bullet}$ test*

*Reagent preparation:* 0.95 mmol/l solution of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, m = 0.00374 g/100 ml). First, this amount is dissolved in 50 ml of dimethyl sulfoxide and after dissolution made up to a volume of 100 ml with ACS water. A 150 µl volume of reagent was incubated with 15 µl of sample. Absorbance was measured at 505 nm for 12 minutes. Output ratio was achieved by the calculation of difference of absorbance at the last ($12^{th}$) minute and at the second minute of the assay procedure.

*2.6.3 Determination of antioxidant activity by the Free Radicals method*

*Reagent preparation*: 5 mL of reaction buffer (100 mmol/l HCl) is diluted with 45 ml of ACS water. To this solution, 100 µl of chlorophyllin is added. After its complete dissolution, 0.25 ml of catalyst is added.

A 150 µl volume of reagent was injected into a plastic cuvette with subsequent addition of a 6 µl sample. Absorbance was measured at 450 nm at the second minute of assay and the last ($12^{th}$) minute. Difference between these two absorbencies was considered as an outputting value and served for the calculation of antioxidant capacity.

### *2.7 Determination of reduced and oxidized glutathione*

Reduced and oxidized glutathione was determined using high performance liquid chromatography with electrochemical detection (HPLC-ED). The chromatographic system consisted of two solvent delivery pumps operating in the range of 0.001–9.999 $ml \cdot min^{-1}$ (Model 582 ESA Inc., Chelmsford, MA, USA), Zorbax eclipse AAA C18 (150 × 4.6; 3.5 µm particle size; Agilent Technologies, Santa Clara, CA, USA) and a CoulArray electrochemical detector (Model 5600A, ESA). The electrochemical detector includes three flow cells (Model 6210, ESA). Each cell consists of four working carbon porous electrodes, each one with auxiliary and dry Pd/H2 reference electrodes. Both the detector and the reaction coil/column were thermostated. The sample (20 µl) was injected using autosampler (Model 542 HPLC, ESA). Samples were kept in the carousel at 8 °C during the analysis. The column was thermostated at 32 °C. Mobile phase consisted of 80 mM TFA (A) and methanol (B). The compounds of interest were separated by the following linear gradient: 0 → 1 min (3% B), 1 → 2 min (10% B), 2 → 5 min (30% B), 5 → 6 min (98% B). Mobile phase flow rate was of 1 ml/min, working electrode potential 900 mV. Time of analysis was 20 min [33-36].

### *2.8 Determination of zinc*

Determination of zinc ions by differential pulse voltammetry was performed using a 797 VA Computrace instrument connected to 813 Compact Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of $0.4mm^2$ was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and platinum electrode was auxiliary. For data processing, 797 VA Computrace software by Metrohm CH was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%). Acetate buffer (0.2 M $CH_3COONa$ + $CH_3COOH$, pH 5) as a supporting electrolyte was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of -1.2 V, end potential -0.7 V, deoxygenating with argon 90 s, deposition 420 s, time interval 0.04 s, step potential 5 mV, modulation amplitude 25 mV, adsorption potential -1.2 V, volume of injected sample: 10 µl, volume of measurement cell 2 ml (10 µl of sample, 1990 µl of acetate buffer) [31,37].

### *2.9 Determination of metallothionein*

Differential pulse voltammetric measurements were performed with 747 VA Stand instrument connected to 693 VA Processor and 695 Autosampler (Metrohm, Switzerland), using a standard cell

with three electrodes and cooled sample holder and measurement cell to 4 °C (Julabo F25, JulaboDE). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 $mm^2$ was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and glassy carbon electrode was auxiliary. For data processing VA Database 2.2 by Metrohm CH was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999 %) saturated with water for 120 s. Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer ($NH_3$(aq) + NH4Cl, pH = 9.6) was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, Eads = 0 V, volume of injected sample: 10 µl, volume of measurement cell 2 ml (10 µl of sample, 1990 µl of Brdicka solution) [38-42].

*2.10 Determination of selenium concentration in the ejaculate*

The concentration of selenium was determined using differential pulse voltammetry by 797 VA Computrace and 889 IC Sample Center (Metrohm, Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of 0.4 $mm^2$ was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and glassy carbon electrode was auxiliary. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999 %) saturated with water for 120 s. The parameters of the measurement were as follows: deposition potential -0.6 V, accumulation time 200 s, pulse amplitude 0.03 V, pulse time 0.05 s, voltage step 0.006 V, voltage step time 0.1 s, sweep rate 0.06 V/s, equilibration time 30 s. Total volume of the measuring vessel was 2 ml (1980 µl of electrolyte and 20 µl of sample). The electrolyte for selenium was prepared by using 0.015 mM ammonium sulfate with addition of copper sulfate; the final concentration of CuSO4 in solution was 0.05 mM. pH of this electrolyte was adjusted to 2.2 using sulfuric acid. The scan was in the range of potentials -0.4 V to -0.9 V and the characteristic peak of selenium was recorded at potential of -0.7 V.

*2.11 Descriptive statistics*

Data were processed using MICROSOFT EXCEL® (USA) and STATISTICA.CZ Version 8.0 (Czech Republic). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). Statistical significances of the differences between MT levels; GSH-Px; Se; Zn; GR; GSH/GSSG; FR; ABTS; DPPH and total number sperms boars were determined using STATISTICA.CZ. Differences with $p < 0.05$ were considered significant and were determined by Student t-test, which was applied for means comparison. The values were based on the differences between the groups supplemented with the selenium dose of 0.6 and 0.3 mg (values in the group 0.6 minus the values in the group 0.3). Values were recalculated to relative percentages with the first sample representing 100 %; other samples were related to this.

## 3. RESULTS

During the experiment the addition of different levels and forms of selenium (organic and inorganic) were evaluated on the basis of the antioxidant status of breeding boars ejaculate. The content of selenium was increased in the ejaculate of animals of G3 group supplemented with the organic form of selenium in the fifth sampling by 81.5 % ($p < 0.05$). In the animals supplemented with inorganic forms of selenium no significant differences were observed in selenium concentration in the ejaculate. The glutathione peroxidase (GSH-Px) activity in group G3 was linearly increased up to 62.0 % ($p < 0.01$) at the end of the experiment. Animals of G4 group had increased levels of GSH-Px too; however, this increase was without statistical significance.

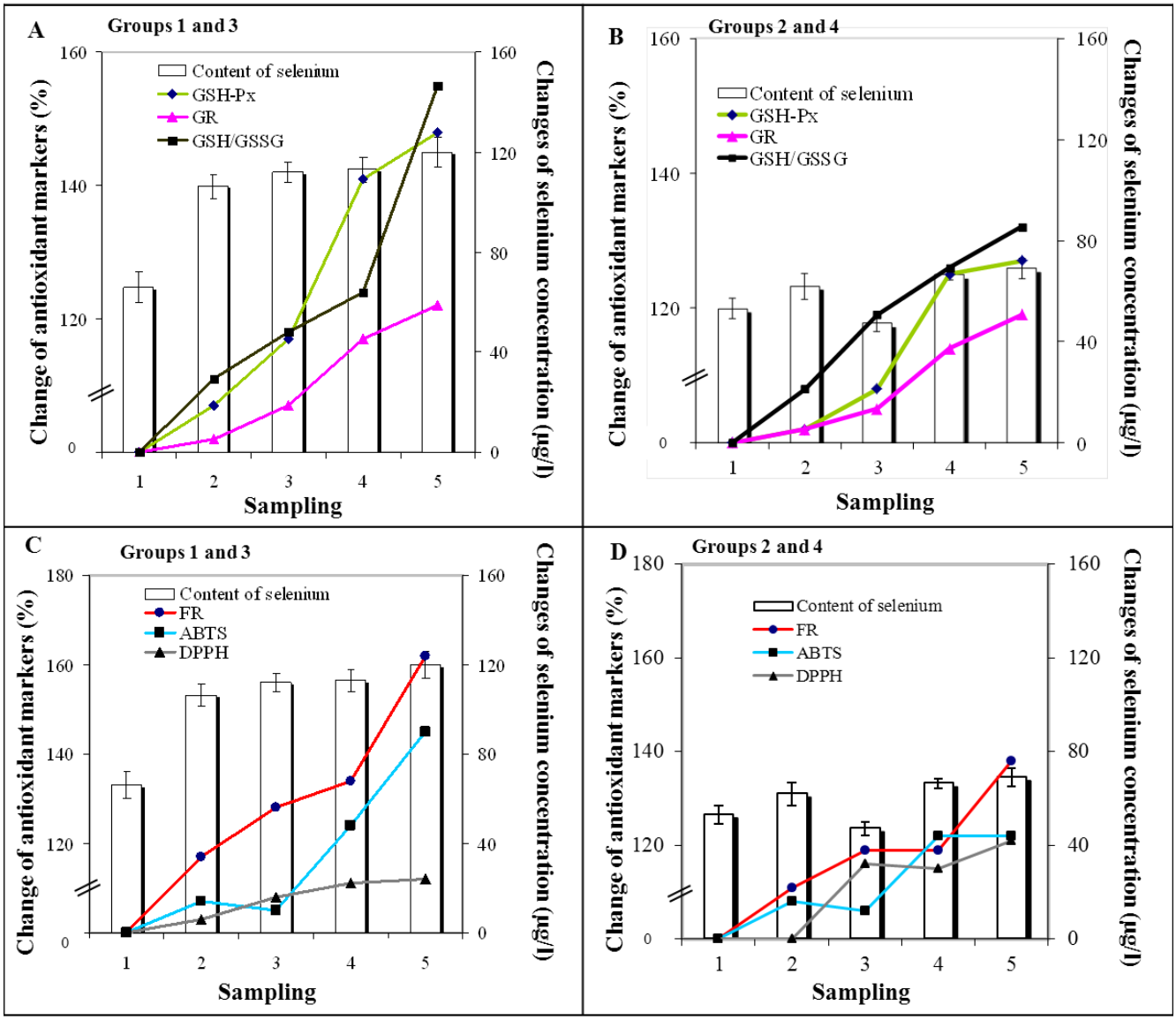


**Figure 1.** Effect of selenium on: (**A, B**) GSH-Px; GR and GSH/GSSG; (**C,D**) antioxidant capacity determined using the FR, ABTS, and DPPH tests. Figures (**A**) and (**C**) are based on the subtraction of values determined in G3 and G1 experimental groups; figures (**B**) and (**D**) are based on the subtraction of values determined in G4 and G2 experimental groups.

The activity of cellular glutathione reductase (GR) was increased all experimental groups of boars, but without statistical evidence too. Higher concentrations were detected in a G3 group supplemented with the organic form of selenium. When evaluating the ratio of reduced (GSH) and oxidized (GSSG) glutathione, significant increase ($P < 0.05$) was found in G3 experimental group (by 55.0 %) in the fifth sampling, as well as in the G4 group of animals, which had increased levels of GSH/GSSG by 31.0 %, however without statistical significance. The antioxidant capacity determined by the Free Radicals test (FR) was the highest in the G3 group, where enhanced to 62.0 % ($p < 0.01$). The G4 group supplemented with the inorganic form of selenium had increased antioxidant capacity in the fifth sampling by 38 % ($p < 0.05$).

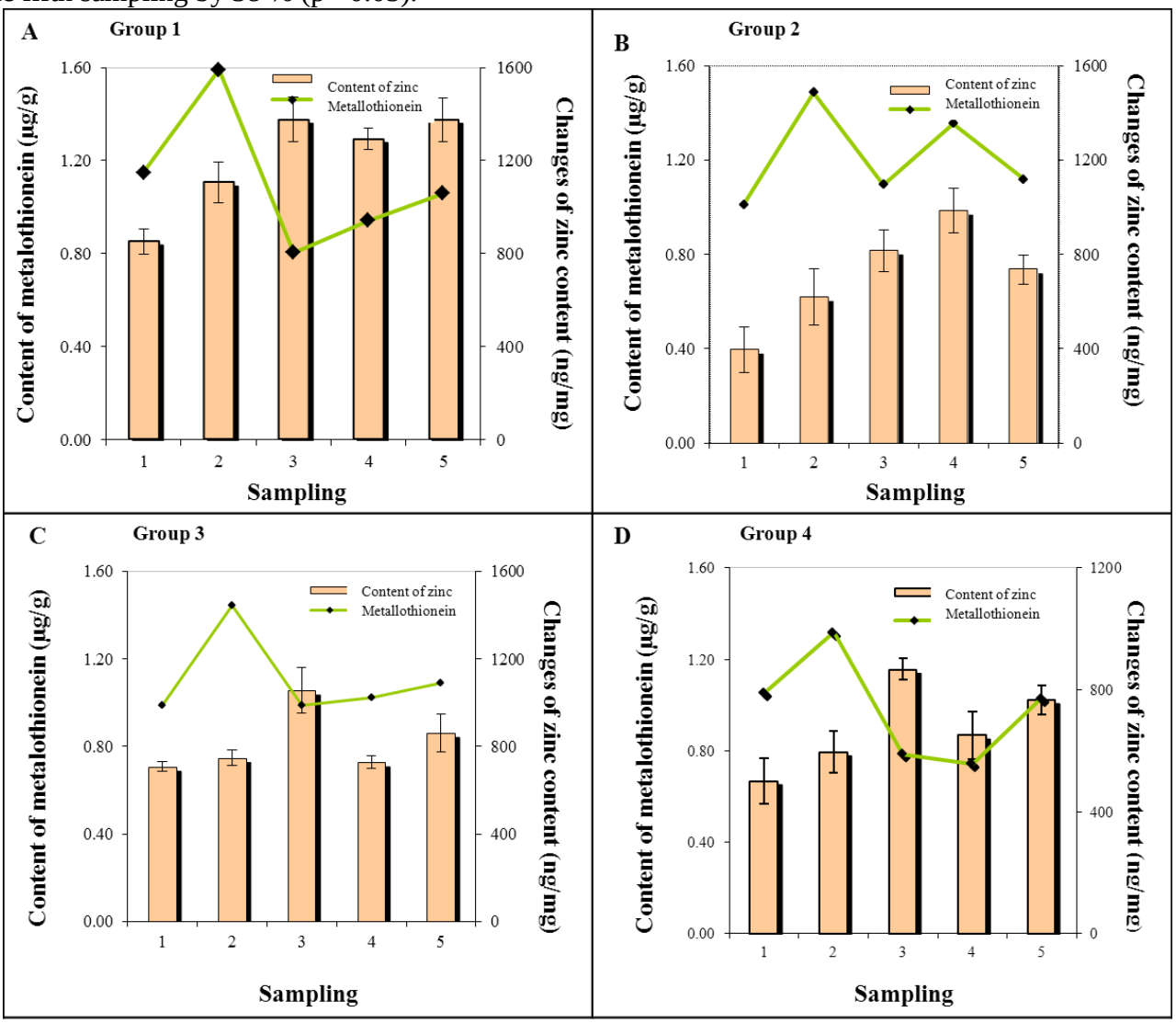


**Figure 2.** Effect of selenium on the zinc and metallothionein levels in the ejaculate of boars: (**A**) Group 1 – 0.3 mg Se/kg FM in the organic form; (**B**) Group 2 – 0.3 mg Se/kg FM in the inorganic form; (**C**) Group 3 – 0.6 mg Se/kg FM in the organic form; (**D**) Group 4 – 0.6 mg Se/kg FM in the inorganic form.

In the determining the antioxidant capacity by the ABTS test, the G3 group gave evidence of a gradual increase, which reached 45.0 % ($p < 0.05$) at the end of the experiment. In the G4 group of boars, the increase by 21.0 % without statistical evidence was observed. Using DPPH test, significant

differences were not detected in any of the experimental groups of animals. Total antioxidant status of boar ejaculate is shown in Fig. 1.

Concentration of zinc in ejaculate was characterized by high variability, its average values ranged from 396.6 to 1376.0 ng/mg. The highest concentration of zinc in the ejaculate was measured in the G1 group of boars supplemented with 0.3 mg Se/kg FM.

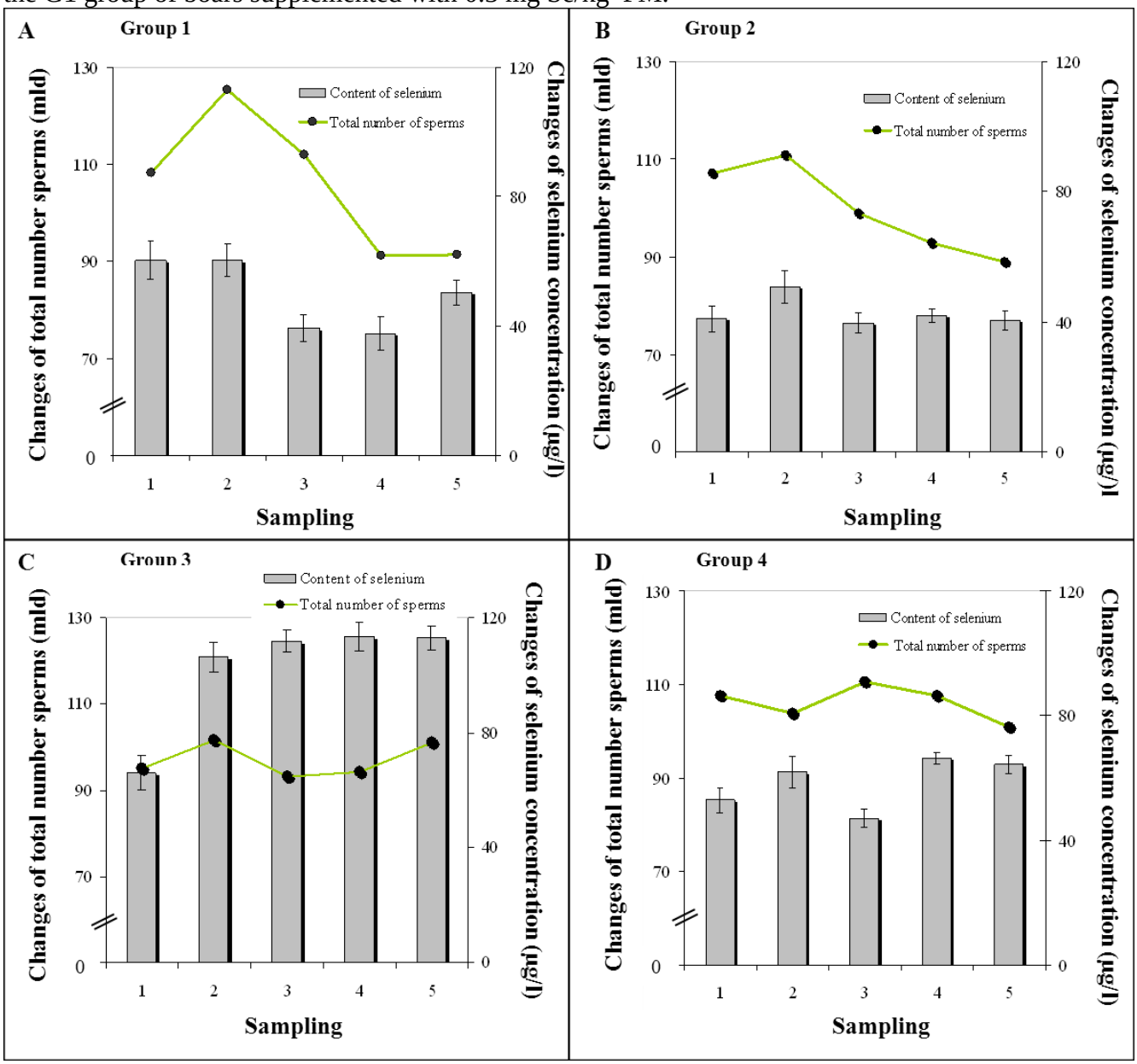


**Figure 3.** Effect of selenium, on the concentration – total number of sperm cells produced by one boar. (**A**) Group 1 – 0.3 mg Se/kg FM in the organic form; (**B**) Group 2 – 0.3 mg Se/kg FM in the inorganic form; (**C**) Group 3 – 0.6 mg Se/kg FM in the organic form; (**D**) Group 4 – 0.6 mg Se/kg FM in the inorganic form.

Increase ($p < 0.05$) between the first and fifth sampling was 61.5 %. Gradual increase ($p < 0.01$) in zinc concentration up to the fourth sampling (by 248.5 % compared to the first sampling) was observed in the second group of animals (G2). The G3 group of boars demonstrated no significant

differences in zinc concentration for the whole duration of the experiment. Animals of G4 group had the highest levels of zinc in the ejaculate of the third sampling (increase by 73.0 % compared to the first sampling). When evaluating the metallothionein (MT) levels, they were identical and highest in all experimental groups in the ejaculate of second collection. Correlation between the concentration of zinc and MT in boars ejaculate has not been established. Results of zinc and MT concentrations in ejaculate of boars are shown in Fig. 2.

When evaluating the sperm cells concentration, the decrease by 13.3 % was detected in the G1 experimental group. In G2 experimental group, the decrease in the ejaculates of the fourth and fifth sampling by 17.3% ($p < 0.05$), respectively 22.7% ($p < 0.001$) compared with the first sampling was well evident. G3 experimental group of boars evidenced no significant changes. The G4 group of animals had decreased concentration of sperm cells by 13.2 % lower at the end of the experiment compared to the beginning of the experiment. Development of the total number of sperm cells produced by a boar per one sampling is shown in Fig. 3.

## 4. DISCUSSION

Marin-Guzman et al. [43] supplemented the diet of experimental groups of boars with the selenium in the concentration of 0.5 mg Se/kg in organic form. Selenium was not increased in the control group of animals. This team of authors found the similar results as in our experimental observations. In the case of treated boars with the higher dose of selenium, GSH-Px activity in ejaculate increased by 114.0 % ($p < 0.01$), in the seminal plasma by 306.5 % ($p < 0.01$) and in sperm by a 68.7 % higher activity compared with the control group of boars. These authors also observed a similar trend in concentration of selenium. The concentration of selenium was more than fourfold higher ($p < 0.01$) in the total ejaculate of experimental animals. During the evaluation of the results, the authors detected for more than twofold higher concentration of selenium ($p < 0.01$) in seminal plasma and sperm cells than in boars without the supplementation of this element in the diet. According to these results, the amount of GSH-Px in seminal plasma of boars increased also with the age of animals. Authors also detected higher amount of sperm cells compared to the control group by 17 %. Similar results have been published by Marin-Guzman et al. [44], who observed increase of number of sperm cells by 53.3 % in the experimental group supplemented by selenium compared to the group without supplementation. However, quality of ejaculate is affected also by other elements. Recently, role of chromium is widely discussed. Nevertheless, its application in practice is complicated and difficult [45]. Another experiment was based on the supplementation of diet (basic diet, 0.15 mg Se/kg FM) with 0.3 mg Se/kg FM in the organic form (first experimental group), the second experimental group was supplemented with selenium (0.3 mg Se/kg FM) in the inorganic form. No significant changes in the markers of the ejaculate quality (motility, number of sperm cells, viability) were found after 84 days of diet supplementation [46]. To the contrary, we detected significantly reduced number of sperm cells in the experimental group of boars supplemented with selenium (0.3 mg/kg/ FM) in inorganic form compared to those supplemented with the same dose of selenium in the organic form. In addition, another team of authors demonstrated that the inorganic form of selenium (sodium selenite) had the

similar effect on the quality of ejaculate as the organic form of selenium [47]. In our experiment, the group without intake of the trace element selenium was missing; but the amount of GSH-Px was not significantly increased in comparison with previous authors. The highest GSH-Px activity and selenium concentration were found in boars, which were supplemented with selenium in organic form at a dose of 0.6 mg/kg of FM. Similar experimental monitoring has been done by Speight et al. [48], whose the diet of three experimental groups of boars supplemented with 0, 0.3 and 0.5 mg Se/kg of FM in the organic form. During the evaluation the activity of GSH-Px, they found linear growth of its activity depending on the amount of selenium supplied in the diet. When evaluating the presence of free radicals by the MDA, no significant difference between the groups has been found. Ebeit [49] also argues that the addition of organic selenium to taps feed doses of 0.3 mg/kg of FM reduced the number of free radicals and decreased lipid peroxidation. Jelezarsky et al. [50] found in the measurement of GSH-Px activity in ejaculate of boars that the highest level of this enzyme was located in the seminar plasma and was more than three times higher compared to spermatozoa. This finding was confirmed by Lasota et al. [51], whose also found approximately one and a half times higher GSH-Px activity in seminal plasma than in spermatozoa. Svoboda et al. [52] compared three different sources of selenium in growing pigs (bacteria *E. faecium* enriched with selenium, selenium-enriched yeast and sodium selenite) at a dose of 0.3 mg/kg of FM. Concentration of selenium in the muscle of pigs that were fed by selenium-enriched bacteria *E. feacium* was comparable with sodium selenite, but were lower ($P < 0.05$) in contrast to selenium enriched yeast. Concentration of selenium and GSH-Px activity in blood serum of individual groups of pigs were similar. Experiments carried out by Patyka et. al. [53]; Silva et al. [54] demonstrated direct correlation between the number of viable sperm cells and GSH-Px activity. Identical conclusion has been published by Brennan et al. [55], who determined that the low selenium dose results in the deterioration of the laboratory markers of ejaculate quality as well as insemination abilities.

Fabrik et al. [56], Mao et al. [57] and Cho et al. [58] monitored concentration of MT in the ejaculate of different animals. They have found no significant correlations between the MT content and the quality of produced sperm cells. This fact was confirmed also in our study. According to Babula et al. [59], concentration of MT is based also on the amount of zinc in organism. By the comparison of MT and zinc in ejaculate, we have not confirmed this fact in our experiment. However, questions about relationship between zinc and MT are very complicated and must be further discussed. Du et al. [60], and Fabrik et al. [56] monitored concentration of zinc in seminal plasma in two groups of boars. First group was characterized by the low quality of ejaculate. Significantly reduced content of zinc in ejaculate ($P < 0.05$) compared to boars with normal values of ejaculate markers quality was detected. In next study, MT was determined in different animal organs. The highest MT content was detected in the reproduction organs, the lowest in liver [61]. Experimental mice were supplemented by zinc in experiment with the focus on MT concentrations in individual tissues and organs. It has been demonstrated that high zinc levels significantly reduced the concentration of MT in blood plasma and mucous membrane of intestine, which results in the stability of intestine microflora [62]. Effect of the high zinc dosages can be used in the supporting of the correct digestion of pigs [63]. Moreover, MT may have under certain conditions also antioxidant capacity [25]. Some studies indicate that zinc may partially eliminate the harmful effect of cadmium, especially production of ROS [64]. Zinc is involved

in the correct development of sperm cells and is important for their correct function [65]. We have not determined correlation between total sperm cells number and concentration of zinc in ejaculate in our experiment.

## 5. CONCLUSIONS

The results of the present study indicate that dietary supplements with 0.6 mg organic Se/kg of feed mixture improve antioxidant potential of breeding boars ejaculate. A lower oxidative stress and higher fertility can be therefore expected. The experimental group of boars supplemented with 0.3 mg Se/kg KS in the inorganic form demonstrated significantly reduced production of sperm cells. Animals supplemented with selenium in the relatively high concentration (0.6 mg Se/kg FM) in the organic form had higher levels of this element in ejaculate. No correlation between MT and zinc has been found.

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