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Effect of parenteral selenium administration on oxidative status of weaned piglets

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Abstract

The aim of the study was to evaluate the effect of additional selenium injection after weaning on the selenium (Se) status of piglets and to find whether the selected dose would be appropriate with respect to the level of oxidative stress. Another goal was to compare the efficacy and safety of sodium selenite and selenopyran as selenium sources for parenteral administration to piglets. Altogether 30 piglets were divided equally into three groups. Piglets in group 1 were injected i.m. with sodium selenite, piglets in group 2 were injected with selenopyran. The dose was 0.42 mg Se/kg body weight for both groups. Piglets in group 3 were given only saline. As expected, the study revealed low Se serum concentrations in weaned piglets. The injection of sodium selenite increased Se serum concentrations but did not have a positive effect on the peroxidase activities. Administration of selenopyran did not influence Se concentrations and gluthation peroxidase activities. The selected dose did not have a significant impact on the level of the oxidative stress. The piglets receiving Se only from the feed achieved comparable gluthation peroxidase activities during the trial. It seems that despite initially low Se concentrations, the physiological requirements for gluthation peroxidase synthesis were met with the feed consumption as the only Se source. The results of the study are important because until now it was unclear whether the selected dose would have negative effects on the organism with respect to the induction of oxidative stress in piglets.

Selenopyran, swine, carbonyl groups, TBARS, antioxidant

Selenium (Se) is an essential nutrient for swine. It has important antioxidant functions primarily through the gluthation peroxidase enzyme (Sunde 1994). Its deficiency can represent a serious problem in swine production. The most common problem is the presence of the mulberry heart disease (Van Vleet et al. 1975). The deficiency is most frequently found in pigs during the first weeks post weaning (Mahan 1996). Upon weaning, a rapid decline in pig serum and tissue Se concentrations occurs (Meyer et al. 1981). Despite the high dietary concentrations of Se in starter diets, its deficiency can still be found on some farms. Sivertsen et al. (2007) found that 54% of the piglets after weaning had plasma Se concentrations below physiological levels. This raises a question whether it would be beneficial for some farms to inject pigs with Se at weaning. Inorganic sodium selenite and organic selenopyran are available for i.m. administration. The use of injective selenopyran could be advantageous because it has low toxicity (Boryaev and Kravchenko 2006). Selenopyran has been used recently for the improvement of the antioxidant status of pig ovaries (Abadjieva et al. 2014). Selenopyran also increased Se content in the sperm of rams (Kistanova et al. 2015).

To our knowledge, there are no objective data yet evaluating the effects of additional selenium injection to piglets after weaning on their selenium and oxidative status. The first aim of the study was to evaluate the effect of additional selenium injection after weaning on

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the selenium status of piglets. The second aim was to find whether the selected dose would have an impact on the level of oxidative stress in piglets. The third aim was to compare the efficacy and safety of sodium selenite and selenopyran as Se sources for parenteral administration to piglets.

Materials and Methods

Experimental design

Altogether 30 weaned piglets were used in the study. They originated from sows of the third parity. The piglets were weaned on day 28. At weaning, the piglets were allotted to three equal groups according to the principle of split litters. Then pigs were moved to a climate-controlled facility and placed in weaning pens situated next to each other. Pigs were individually marked with a plastic badge in the right ear.

The feed of sows contained 0.3 mg Se/kg in the form of sodium selenite. Starting from 10 days of age the piglets had *ad libitum* access to the pre-starter diet. This diet contained 0.4 mg Se/kg, of which 0.3 mg Se/kg was present as sodium selenite and 0.1 mg Se/kg was in the form of selenium enriched yeast. The diet was administered till 2 weeks after weaning. Then the piglets were offered the starter diet with 0.3 mg Se/kg as sodium selenite. The diets were produced by De Heus a.s., Marefy, Czech Republic.

Piglets in group 1 were injected intramuscularly with oil solution of selenopyran 10 days after weaning at a dose of 0.42 mg Se/kg live weight. Selenopyran was provided by Penza State Agricultural Academy, Russia. Piglets in group 2 were injected with sodium selenite in water solution at a dose of 0.42 mg Se/kg live weight. Piglets in group 3 were injected saline and served as the control group.

The study was approved by the Ethics Committee of the University of Veterinary and Pharmaceutical Sciences Brno.

Sampling and analysis

Blood samples were taken before the injection of selenium, 48 h after the administration, and then 7, 14, and 21 days post treatment. The piglets were weighed at the same intervals. Blood was collected from their vena cava cranialis. After sampling, part of the blood sample was coagulated in special sterile tubes with serum accelerators for determination of Se concentrations. Heparin was used as an anticoagulant for determination of the antioxidant status and blood biochemistry.

Selenium concentrations were determined by the Solar 939 AA spectrometer (Unicam, UK) using the hydride AAS technique. The samples were prepared by mineralization in a closed system using a microwave (MLS-1200, Milestone, Italy) digestion technique with HNO_3 and H_2O_2. After mineralization, the samples were evaporated and the mineral residue dissolved in water to which 20% HCl was added.

Vitamin E concentrations in serum were determined fluorometrically according to Bouda et al. (1980) using fluorescence spectrophotometer 204 (Perkin-Elmer, USA).

The activity of GPx (gluthation peroxidase) in plasma was determined spectrophotometrically and calculated from the rate of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidation by the reaction with gluthation reductase (GR) at 340 nm (Flohe and Gunzler 1984). The specific activity of was expressed as the nmol of NADPH consumption per min per mg of protein.

The concentration of thiobarbituric acid reactive substances (TBARS) in plasma was measured spectrophotometrically at 535 nm using the Varioskan flash spectral scanning multimode reader (Thermo Fisher Scientific Inc., USA) according to the method described by Ohkawa et al. (1979) with modifications by Lushchak et al. (2005). The TBARS concentrations are expressed as nanomoles per milliliter of plasma.

Trolox equivalent antioxidant capacity (TEAC) was determined in plasma following the protocol of Re et al. (1999) and measured spectrophotometrically at 734 nm using a Varioskan flash spectral scanning multimode reader (Thermo Fisher Scientific Inc., USA). The TEAC values were expressed as millimoles of trolox as external standard per milliliter of plasma.

Carbonyl derivatives of proteins (CP) were detected spectrophotometrically by reaction with 2,4-dinitrophenylhydrazine (DNPH) using the Varioskan flash spectral scanning multimode reader (Thermo Fisher Scientific Inc., USA) according to the method described by Lenz et al. (1989) and modified by Lushchak et al. (2005). The CP are quantified as nanomoles of CP per plasma protein miligram. Plasma protein concentrations in guanidine chloride solution for CP were determined by the Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) using bovine serum albumin as a standard (Smith et al. 1985).

The ceruloplasmin activity in plasma was analysed according to Ceron and Martinez-Subiela (2004) with slight modifications using the Varioskan flash spectral scanning multimode reader (Thermo Fisher Scientific Inc., USA). Results were expressed as the amount of the absorbance increase per min × 10,000. Ferric reducing ability of plasma (FRAP) was measured on a biochemical analyzer Konelab 20i according to Benzie and Strain (1996).

The other plasma biochemical indices such as total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH-L), creatine kinase (CK), and creatinine (CREAT) were determined using the biochemical analyzer Konelab 20i and commercial kits (Biovendor, Czech Republic).

Statistical analysis

Statistical assessment was carried out using Statistica for Windows 8.0. software (Czech Republic). Data were tested for normality (Kolmogorov-Smirnov test). Data did not satisfy normal distribution, therefore, nonparametric Kruskall-Wallis test was used to test the differences between the groups. Moreover, the data were subjected to two-way ANOVA with treatment (control, sodium selenite, and selenopyran) and day of sampling (day 0, 2, 7, 14 and 21) as the main effects along with their interactions. Significant differences in all possible pairs of groups were evaluated using post-hoc Tukey-HSD test. Significance was accepted at $P < 0.05$. All data are reported as mean ± standard deviation.

Results

Serum Se concentration

The results are presented in Fig. 1.

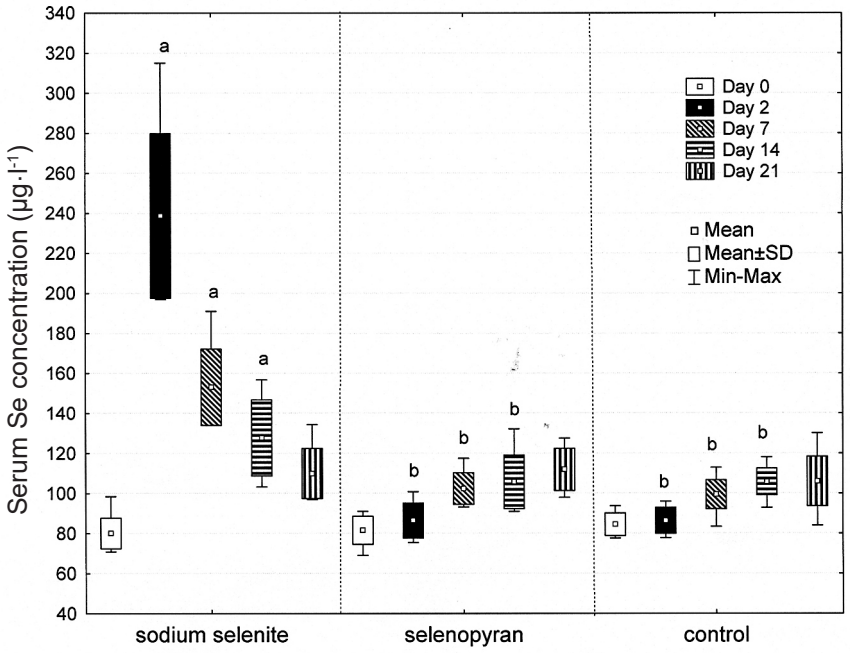

Fig. 1. Selenium concentration of weaned piglets administered different forms of selenium
The results are expressed as mean ± standard deviation. Day 0 means the time of application. Significant differences ($P < 0.05$) between groups are indicated by different alphabetic superscript.

In the control group of our study, the mean serum selenium values appeared to increase from day 0 to day 21, however, the differences were not significant.

Sodium selenite injection at a dose of 0.42 mg Se/kg caused a significant increase of selenium serum concentrations on day 2 (two days after application). These concentrations declined thereafter but continued to be higher compared to the control group during the first two weeks after treatment.

Serum Se concentrations after the injection of selenopyran remained comparable to the control group in all the periods of the study. In contrast to the control group, there was a significant increase of serum Se in the selenopyran group from day 0 to 21.

Oxidative status

The results of TBARS and CP concentrations are presented in Figs 2 and 3.

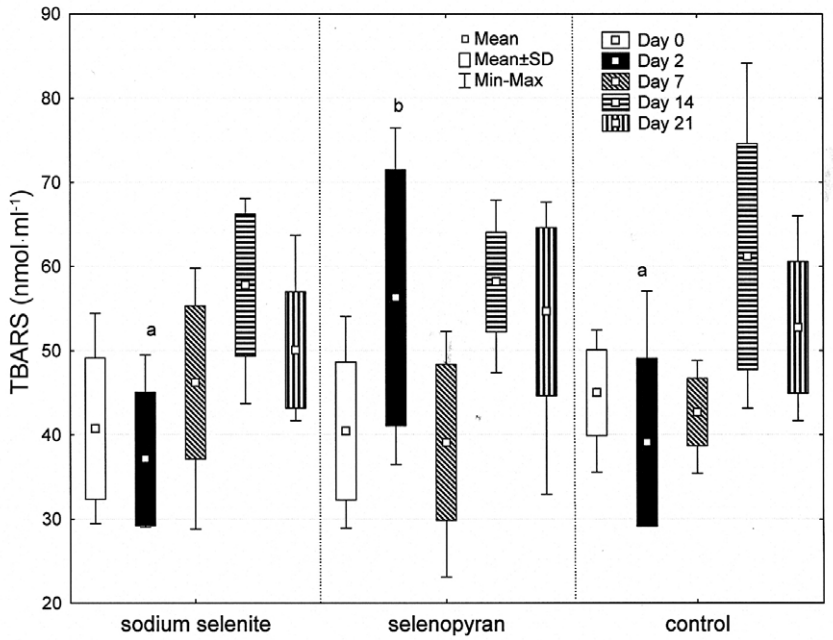

Fig. 2. Thiobarbituric acid reactive substances concentration of weaned piglets administered different forms of selenium
The results are expressed as mean ± standard deviation. Day 0 means the time of application.
Significant differences ($P < 0.05$) between groups are indicated by different alphabetic superscript. TBARS - thiobarbituric acid reactive substances

The other results (vitamin E, GPx, FRAP, TEAC, ceruloplasmin) are presented in Table 1.

In the sodium selenite group, CP concentrations were found to be higher compared to the control group on day 2. In the selenopyran group, TBARS concentrations were higher than control two days after injection. One week after treatment the values returned to normal, i.e. they were statistically comparable to the control group. There was also an increase of protein oxidation in the selenopyran group. Compared to control, the values of CP remained to be higher in all the periods of the trial. No other differences in the oxidative status were found between the groups in any period of the trial.

Blood biochemistry

The results are presented in Table 2. In the selenopyran group, LDH-L activities were lower and creatinine concentrations higher compared to control seven days after treatment. No other significant differences in the studied biochemical indices were found between the groups in any period of the trial.

Body weight

The results are presented in Table 3. No significant differences in the achieved body weights were noted between the groups in any period of the trial.

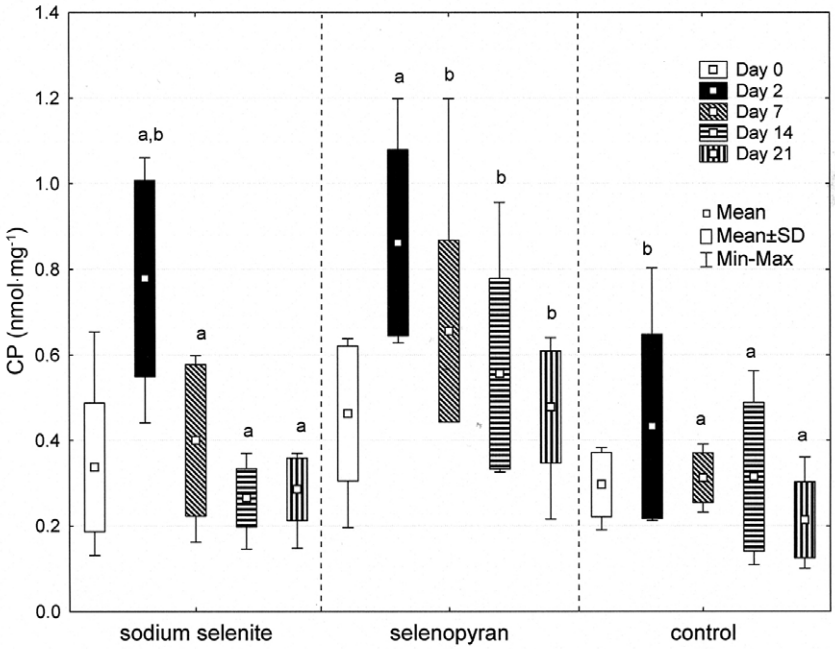

Fig. 3. Carbonyl protein concentration of weaned piglets administered different forms of selenium
The results are expressed as mean ± standard deviation. Day 0 means the time of application.
Significant differences ($P < 0.05$) between groups are indicated by different alphabetic superscript. CP - carbonyl protein

Discussion

Ullrey (1987) and Mahan (1991) declare selenium serum concentrations ranging between 80 and 150 µg/l as normal in pigs. Blood and Radostits (1989) stated that normal concentrations should be even higher, i.e. above 120 µg/l serum. Our results confirm that despite high dietary concentrations of Se in starter diets, its deficiency can still be found on some farms. Ten days after weaning, the mean Se concentrations were around 80 µg/l. We found 33% of piglets with Se concentrations below 80 µg/l. This is in line with the findings of Sivertsen et al. (2007) who reported plasma Se concentrations below 80 µg/l in 54% of weaned piglets, and concentrations of 60 µg/l or less in 19% of weaned piglets. In pig production, selenium is added routinely to starter diets at a concentration of 0.3–0.4 ppm (Sivertsen et al. 2007). The reasons why serum concentrations are low after weaning despite dietary selenium supplementation could be attributed to the following facts. At weaning, a decline in pig serum and tissue Se concentrations occurs (Meyer et al. 1981). Piglets usually eat little during the first days after weaning (Bruininx et al. 2002). The low food intake predisposes them to gastrointestinal disorders and weight loss (Madec et al. 1998).

The increase of serum Se concentrations in the control group was not significant. The initial suboptimal concentrations (20% piglets) were eliminated as a Se concentration below 80 µg/l was not found in any piglet at the end of trial. This indicates that the deficit was supplemented from the feed.

Table 1. Oxidative status of weaned piglets administered different forms of selenium.

	Study day	Vitamin E [µmol/l]	GSH – PX [nmol NADPH/min/ml]	FRAP [µmol/l]	TEAC [µM/ml]	Ceruloplasmin [ΔA/min × 10^4]
Sodium selenite	0	2.42 +/- 0.58	577.45 +/- 190.54	201.92 +/- 36.20	0.66 +/- 0.08	370.26 +/- 163.30
	2	2.27 +/- 0.95	450.13 +/- 121.47	224.37 +/- 28.98	0.69 +/- 0.14	288.04 +/- 100.46
	7	3.01 0.71	476.30 +/- 103.47	268.35 +/- 33.77	0.72 +/- 0.16	293.85 +/- 133.14
	14	3.08 +/- 0.95	545.74 +/- 79.22	282.94 +/- 35.14	0.86 +/- 0.07	292.33 +/- 86.29
	21	2.55 +/- 0.48	642.01 +/- 86.77	212.76 +/- 26.12	0.73 +/- 0.09	271.11 +/- 65.30
Selenopyran	0	2.43 +/- 0.58	557.87 +/- 105.73	218.95 +/- 19.62	0.61 +/- 0.12	440.73 +/- 129.71
	2	2.73 +/- 0.51	496.04 +/- 82.54	243.01 +/- 28.17	0.67 +/- 0.17	396.57 +/- 109.87
	7	3.47 +/- 0.75	472.41 +/- 91.64	272.69 +/- 33.66	0.64 +/- 0.19	288.80 +/- 86.46
	14	3.28 +/- 0.68	539.14 +/- 58.66	273.47 +/- 34.06	0.81 +/- 0.07	325.33 +/- 84.42
	21	2.99 +/- 0.86	616.26 +/- 83.88	222.74 +/- 31.64	0.70 +/- 0.08	303.47 +/- 118.86
Control	0	2.18 +/- 0.61	596.22 +/- 107.77	225.74 +/- 35.41	0.66 +/- 0.12	336.47 +/- 84.89
	2	2.75 +/- 0.84	465.08 +/- 62.76	243.61 +/- 34.07	0.67 +/- 0.11	377.63 +/- 104.68
	7	3.03 +/- 0.90	477.49 +/- 66.86	279.71 +/- 54.85	0.72 +/- 0.11	261.44 +/- 70.02
	14	3.37 +/- 0.81	577.14 +/- 84.93	285.94 +/- 44.08	0.85 +/- 0.07	298.53 +/- 55.06
	21	2.93 +/- 0.55	721.34 +/- 139.82	219.05 +/- 38.56	0.70 +/- 0.04	265.33 +/- 70.01

The results are expressed as mean ± standard deviation. Day 0 means the time of application.
FRAP- ferric reducing ability of plasma, GPx-gluthation peroxidase, TEAC-trolox equivalent antioxidant capacity.

Table 2. Blood biochemistry of weaned piglets administered different forms of selenium.

	Study day	TP [g/l]	ALT [µkat/l]	AST [µkat/l]	ALP [µkat/l]	LDH- L [µkat/l]	CK [µkat/l]	Urea [mmol/l]	CREAT [µmol/l]
Sodium selenite	0	45.33 +/- 3.08	0.90 +/- 0.12	0.70 +/- 0.14	14.02 +/- 7.28	14.02 +/- 1.77	7.83 +/- 2.09	1.26 +/- 0.59	102.54 +/- 22.85
	2	43.20 +/- 5.59	1.10 +/- 0.15	0.91 +/- 0.24	14.02 +/- 7.60	14.47 +/- 2.02	11.79 +/- 6.19	0.86 +/- 0.29	87.30 +/- 17.01
	7	46.71 +/- 10.67	1.32 +/- 0.56	1.03 +/- 0.17	14.68 +/- 7.15	16.40 +/- 2.86	15.26 +/- 5.28	1.32 +/- 0.91	91.30 +/- 18.44
	14	48.65 +/- 5.12	1.82 +/- 0.44	1.27 +/- 0.15	6.83 +/- 5.50	15.64 +/- 2.73	15.86 +/- 4.48	1.62 +/- 0.82	96.88 +/- 11.14
	21	53.07 +/- 5.31	1.93 +/- 0.63	1.01 +/- 0.17	12.66 +/- 5.70	13.85 +/- 2.29	25.44 +/- 21.00	2.21 +/- 1.04	92.85 +/- 15.69
Selenopyran	0	45.74 +/- 5.53	0.81 +/- 0.12	0.85 +/- 0.23	8.06 +/- 1.94	14.26 +/- 3.73	7.03 +/- 2.65	1.12 +/- 0.60	102.99 +/- 7.64
	2	42.99 +/- 2.44	1.11 +/- 0.21	0.90 +/- 0.19	12.99 +/- 3.72	14.12 +/- 2.71	11.80 +/- 6.70	1.35 +/- 0.80	83.96 +/- 10.31
	7	49.05 +/- 5.16	1.81 +/- 0.52	1.15 +/- 0.43	9.08 +/- 3.63	13.42 +/- 4.22[a]	18.15 +/- 12.47	1.27 +/- 0.34	107.39 +/- 17.80[a]
	14	48.44 +/- 3.51	1.85 +/- 0.45	1.31 +/- 0.40	9.90 +/- 3.66	16.88 +/- 4.11	14.89 +/- 6.03	1.76 +/- 0.48	108.41 +/- 9.42
	21	53.97 +/- 4.10	1.94 +/- 0.62	0.95 +/- 0.24	21.65 +/- 7.03	14.67 +/- 2.50	30.95 +/- 27.59	2.41 +/- 0.25	100.26 +/- 9.52
Control	0	47.03 +/- 6.11	0.91 +/- 0.21	0.73 +/- 0.15	8.09 +/- 3.33	13.42 +/- 2.23	7.29 +/- 2.87	1.29 +/- 0.57	102.97 +/- 15.68
	2	45.68 +/- 3.45	1.04 +/- 0.25	0.93 +/- 0.23	11.59 +/- 5.57	14.04 +/- 1.74	10.66 +/- 3.03	1.23 +/- 0.57	88.78 +/- 14.73
	7	47.28 +/- 4.13	1.73 +/- 0.54	1.36 +/- 0.33	10.12 +/- 5.27	17.62 +/- 2.42[b]	20.26 +/- 7.74	1.05 +/- 0.23	86.91 +/- 5.47[b]
	14	49.80 +/- 3.97	2.46 +/- 0.92	1.40 +/- 0.41	10.27 +/- 4.39	15.60 +/- 2.59	14.65 +/- 5.80	1.894 +/- 0.5	105.77 +/- 16.04
	21	52.92 +/- 3.89	2.60 +/- 1.06	1.17 +/- 0.49	15.60 +/- 9.46	11.94 +/- 5.51	30.69 +/- 23.81	2.13 +/- 0.76	94.52 +/- 8.72

The results are expressed as mean ± standard deviation. Day 0 means the time of application. Significant differences ($P < 0.05$) between groups are indicated by different alphabetic superscript. ALP - alkaline phosphatase, ALT - alanine aminotransferase, AST - aspartate aminotransferase, CK - creatine kinase, CREAT - creatinine, LDH-L - lactate dehydrogenase, TP - Total protein.

Table 3. Body weight (kg) of weaned piglets administered different forms of selenium.

	Weight [kg]		
Study day	Sodium selenite	Selenopyran	Control
0	9.8	9.6	9.7
	+/-	+/-	+/-
	2.2	1.6	2.1
7	11.9	12.4	12.6
	+/-	+/-	+/-
	2.7	2.1	2.7
14	14.5	14.3	14.9
	+/-	+/-	+/-
	2.9	2.3	3.3
21	19.0	18.9	19.5
	+/-	+/-	+/-
	3.7	3.1	3.7

The results are expressed as mean ± standard deviation. Day 0 means the time of application.

In our study, sodium selenite injection at a dose of 0.42 mg Se/kg caused a significant increase of serum Se concentrations two days after treatment. These concentrations declined thereafter but remained to be higher compared to the control group during the first two weeks after treatment. This indicates a rapid utilization of Se from this inorganic source.

Serum Se after the injection of selenopyran was comparable to the control group in all the periods. This indicates a slower utilization of Se from this source. Selenopyran is soluble in oils but not in water and hence has a longer retention time, thus serving as a kind of "Se depot" in the organism, releasing Se slowly based on the needs of the organism. Selenopyran is supposed to be transferred as a constituent part of lipoproteins (Boryaev et al. 2006). In general, when a lipid emulsion is given intramuscularly it is not transported directly to the blood. The majority of LE droplets enter the lymphatic system and eventually the blood circulation where they are taken up rapidly by the circulating monocytes for clearance by the reticuloendothelial cells (through organs such as the liver, spleen and bone marrow) (Tamilvavan 2004). All these factors could have contributed to the low serum selenium concentrations after selopyran treatment under the conditions of our study.

The TBARS are well-known secondary products of lipoperoxidation (Tsaknis et al. 1998). One day after selenopyran administration there was a transitory increase of TBARS concentration which indicates lipid peroxidation. One week after treatment the values returned to normal. There was also an increase of protein oxidation in the selenopyran group. The values of CP were higher in all the periods of the trial. Whereas oxidized proteins are degraded within hours or days, secondary products of lipid peroxidation are detoxified generally within minutes (Grune et al. 1995; Siems et al. 1997; Dalle-Donne et al. 2003). In our study, there was a slight increase in oxidative stress after selenopyran administration but the products of lipid peroxidation are likely to be quickly removed. Oxidation products of proteins are relatively stable and remained elevated for the rest of the experiment.

The sodium selenite injection in our study caused an increase of serum Se concentrations but did not affect GPx activities compared to the control group of piglets. It has been documented that serum GPx activities reach a plateau as the dietary Se concentration rises

with a minimal increase at higher supplemental Se concentrations (Mahan et al. 1999). Apparently, despite the fact that Se concentrations were initially low they still met the physiological requirements for Gpx synthesis under the conditions of our study.

The selected dose of 0.42 mg Se per kg was not a random choice. It was based on previous studies of other authors (Van Fleet et al. 1974, 1975). These researchers found that after sodium selenite injection, toxicity and deaths occur in pigs when the dose exceeds 0.9 mg Se/kg body weight. Lower dosage within the range of 0.44–0.73 mg Se/kg body weight does not cause death. However, they found a significant increase of plasma glutamate oxaloacetate transaminase (GOT) and creatine kinase (CK) activities when the dose of 0.72 mg Se/kg body weight was given. It was probably related to the presence of skeletal myodegeneration. The effect of the lower dose (0.44 mg Se/kg body weight) on plasma enzymes was not tested in their study. Also, modern indices that are now available for the evaluation of oxidative stress were not considered in the respective studies.

Therefore, it remains unclear whether the dose of 0.42 mg Se/kg that we used in our study would have any negative effects on the piglet organism with respect to the induction of oxidative stress.

Sodium selenite is a compound with a prooxidative potential (Spallholz 1994). The use of high dietary concentration of sodium selenite in our study did not result in increased lipid oxidation and did not influence negatively other biochemical indices. This can be explained by the fact that Se from sodium selenite builds up in tissues only up to physiological levels, with any excess being excreted in urine (Lindberg and Lannek 1965).

It can be concluded that the additional injection of sodium selenite after weaning increased serum Se concentrations but did not have any positive effect on the GPx activities. Administration of selenopyran did not influence Se concentrations or GPx activities.

The selected dose of 0.42 Se can be regarded as safe because it did not have a significant impact on the level of the oxidative stress and other biochemical indices.

The piglets that were receiving Se only from the feed achieved comparable GPx activities during all the periods of the trial. Apparently, despite the fact that the Se concentrations were initially low, the physiological requirements for GPx synthesis could be met with the use of Se from the feed as the only source.

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