Effects of dietary organic selenium supplementation on selenium content, antioxidative status of muscles and meat quality of pigs

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ABSTRACT: The effects of feeding a high level of organic selenium on the level of selenium, antioxidative status of *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (MSM) and meat quality of pigs (defined on malignant hyperthermia status, DNA based test) were investigated. Treatments consisted in supplementation of organic selenium (0.3 mg Se/kg diet) for the last 97 days to finishing pigs (basic diet with 0.18 mg Se/kg diet) before slaughter. MLLT was further examined for pH (45 min, 24 h), colour and conductivity (24 h), drip loss (48 h) and myofibrillar fragmentation index (MFI, 5 days). Chemical composition (protein, intramuscular fat) was also estimated. Concentration of Se (spectrofluorometric method) and antioxidative status (rate of oxidation by stimulation with Fe^{2+}/ascorbate, production of malondialdehyde – MDA) were estimated in muscle samples obtained *post mortem*. The level of selenium was more than twice higher ($P < 0.05$) in muscles from pigs treated with higher selenium than in controls (0.377 vs. 0.922 mg/kg – MLLT and 0.377 vs. 0.836 mg/kg – MSM). The rate of oxidation was positively ($P < 0.05$) influenced by Se supplementation. Tendencies to lower drip losses were observed in MLLT of pigs supplemented with Se but the differences were not significant ($P > 0.05$). We concluded that dietary organic Se supplementation (0.3 mg Se/kg diet) to basic diet (0.18 mg Se/kg diet) of finishing pigs significantly increased the selenium concentration and improved the antioxidative status of muscle tissue.

Keywords: pigs; selenium; antioxidative status; meat quality

Commercial organic selenium (Se) is now available from several sources in the concentrated form, and the evaluation of the long-term feeding of high levels from an organic Se source was made in several experiments on pigs (Suomi and Alaviuhkola, 1992; Mahan and Parret, 1996; Mahan *et al*., 1999; Kim and Mahan, 2001; Krska *et al*., 2001; Nürnberg *et al*., 2002). It was observed that dietary Se levels >5 ppm were toxic when fed to growing-finishing pigs (Goehring *et al*., 1984) but selenosis seemed to be more severe when the inorganic Se form was provided (Kim and Mahan, 2001). Both daily gains (body weight) and feed intake were shown to be reduced when a dietary Se level increased, particularly when diets were >5 ppm Se (Kim and Mahan, 2001). Tissue (loin, liver, kidney and hoof) Se concentrations increased when pigs were fed organic Se compared with the inorganic Se source, and each tissue increased in Se concentration as the dietary Se level increased (Kim and Mahan, 2001).

Both vitamin E and organic Se are essential nutrients that are integral components of the antioxidant defence system of cells and tissues and have recognized antioxidant properties (Hoekstra, 1975; Walsh *et al*., 1993; Spallholz, 1994). Sodium selenite may also act as a prooxidant, particularly at high dietary levels (Seko *et al*., 1989), whereas selenomethionine does not possess these properties (Spallholz, 1994).

Selenium was earlier identified as an integral part of the enzyme glutathione peroxidase (GSH-Px), which destroys lipid peroxides and functions by protecting the cell membranes against peroxidative damage (Hoekstra, 1975). GSH-Px activity is consid-

ered one of the best indices of selenium status (for a review see Milad and Kovac, 1998). The effects of organic and inorganic Se on pigs have been studied frequently (Goehring *et al*., 1984; Mahan and Parret, 1996; Mahan *et al*., 1999; Kim and Mahan, 2001; Krska *et al*., 2001; Nürnberg *et al*., 2002). The results indicated that the major carcass measurements did not seem to be affected by the dietary Se levels or sources. The inclusion of inorganic Se in the diets of growing-finishing pigs seemed to have a detrimental effect on some pork quality parameters, whereas the organic Se source did not (Mahan *et al*., 1999). Selenium in meat of pigs may also contribute to the solution of a sufficient supply of this element to the human organism (Koutnik and Ingr, 1998).

The objective of this study was to further evaluate the effects of dietary administration of organic selenium on the level of selenium, antioxidative status in muscles and meat quality of malignant hyperthermia (MH) defined pigs.

MATERIAL AND METHODS

Animal, diets and sampling procedure

Twenty Large White and final hybrids were used in this experiment, including 10 gilts and 10 castrates. The *RYR1* genotype (malignant hyperthermia status – MH) of these animals was determined by a DNA based test (Genetic Department, RIAP Nitra) described previously (Bauerova *et al*., 1999). To create homogeneous groups as for the frequency of occurrence of mutation on *RYR1* gene and the sex of pigs two groups were formed with 6 normal and 4 heterozygotes on MH and with equal number of gilts and castrates. The pigs (average live weight of 35 kg) were penned and fed in boxes at breeding cooperative farm (PD Devio Nove Sady). Pigs were fed a low vitamin E (30.9 mg/kg) and selenium (0.18 mg/kg) basal diet (Table 1). Animals of group Sel received the basal diet supplemented with 0.3 mg organic Se/kg diet. Feeding (Schauer technology) and water were *ad libitum*. The facilities met the requirements of the animal care. Animals were stunned, slaughtered and exsanguinated in the slaughterhouse of RIAP Nitra (transportation about 10 km) with the mean live weight of 105 kg. Following slaughter, the carcasses were chilled at 4°C for 24 h, and then the *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (MSM) were removed from each carcass. A portion of the sample was used immediately and the remaining sample was wrapped in aluminium foil and stored in a refrigerator at 4°C for 5 days.

Table 1. Composition of the basic diet

Feed composition	
Wheat (%)	35
Barley (%)	32
Soybean meal (%)	16
Oat (%)	8
Wheat meal (%)	4
Mineral and vitamin mix (%)	3
Meat and bone meal (%)	2
Chemical composition	
Crude protein (%)	17.5
Crude fat (%)	2.3
Crude fibre (%)	4.6
Ash (%)	8.2
Vitamin E (mg/kg)	30.9
Lysine (mg/kg)	9.1
Threonine (mg/kg)	6.4
Methionine (mg/kg)	2.8
Tryptophan (mg/kg)	2.2
Selenium (mg/kg)	0.18

Methods and statistical analyses

The quantitative determination of selenium content in muscles was estimated by the spectrofluorometric method (Spallholz *et al*., 1978) (provided by Institute of Animal Physiology, Slovak Academy of Sciences, Košice).

To evaluate the stability of skeletal muscle lipids against stimulated lipid peroxidation, a determination of thiobarbituric acid reactive substances (TBARS) was performed. To stimulate lipid peroxidation, 3 ml of muscle homogenates were incubated in a mixture of ascorbate/Fe^{2+} for different time (0, 30 min) intervals at 37°C (Lahucky *et al*., 2001). The absorbance at 535 nm was determined. Standard malondialdehyde (MDA) was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane and the results were expressed as mg MDA/g muscle.

The pH value of the carcass (MLLT – between 13th and 14th rib) was determined in 45 min *post mortem* using the combined pH electrode (Ingold). Colour was measured by means of spectrophotometer (Specol, Germany) at 580 nm as external reflectance. Total protein and intramuscular fat were measured by the Infratec-Analyser. Drip loss analysis was made according to Honikel (1998). The myofibrillar fragmentation index (MFI) was determined as described by Hopkins *et al*. (2000).

Statistical analyses were calculated as mean values and standard deviations (one-way A-NOVA) and differences were evaluated by Tukey's test (University of Agriculture, Nitra, Slovakia).

RESULTS AND DISCUSSION

The basal level of Se in muscles (Table 2, Figure 1) is comparable with the results reported by Driskell *et al*. (1997) using a fluorometric method and also with Koutnik and Ingr (1998) using atomic absorption for the determination of selenium content in pork. Tissue Se concentrations increase when higher dietary Se levels are provided. As was shown (Koutnik and Ingr, 1998), the Se content in the muscles of pigs (Seghers) was significantly higher when feed with an addition of *Chlorella vulgaris* was provided. More than twice higher Se concentrations in muscles (MLLT, MSM) were received when additional organic Se was supplemented to diets for pigs (Table 2, Figure l). Our findings are comparable with the results when Se-enriched yeast was fed to finisher pigs (Mahan and Parret, 1996). The results reported by Mahan and Parret (1996) suggested that a higher retention of Se occurred when the organic Se source was fed compared with sodium selenite. Our and other studies deal with the concentrations of selenium in pig meat which may contribute to the solution of a sufficient supply of this element into the human organism. The FDA (1992) indicated that the terms "high", "rich in", or "major source of" should be used when a serving of food (in the case of meats, 100 g) contains 20% or more of the Reference Daily Intake (also referred to as Daily Value). Supplementation with organic Se source in a pig diet is one of the possibilities how to solve problems of sufficient supply of Se in human nutrition.

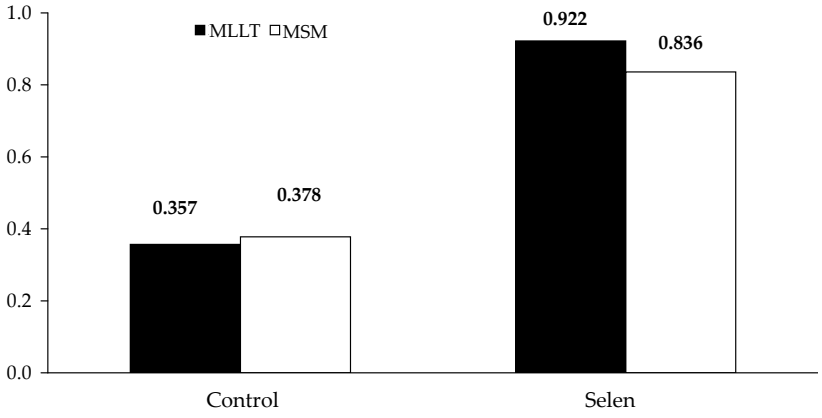

Figure 1. Comparison of selenium ratio in meat dry matter in MLLT and MSM of the individual pig groups with selenium supplementation (mg/kg)

Table 2. Effects of dietary organic selenium supplementation on the concentration of selenium (mg/kg) in *post mortem* samples of *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (MSM) in pigs

Trait	Group C		Group Sel	
	mean	± SEM	mean	± SEM
m. longissimus lumborum et thoracis	0.357	0.016	0.922[b]	0.023
m. semimembranosus	0.377	0.020	0.836[b]	0.034

Group C = control group (standard diet A_1, OS–3, OS–6); (Se 0.18 mg/kg)
Group Sel = group with organic selenium (supplementation of selenium 0.3 mg/kg)
[b]$P < 0.01$

The most important metabolic role of selenium in mammalian species is its function in the active site of the selenoenzyme glutathion peroxidase and this enzyme, together with superoxide dismutase and catalase, protects cells against damage caused by free radicals and hydro- or lipoperoxides (Flohe, 1997). Vitamins E and Se, through the action of the selenoprotein glutathione peroxidase, have important antioxidant functions within cells as a part of the cellular antioxidant defence system (Walsh *et al*., 1993). Lipid peroxidation can be induced and enhanced by employing systems containing prooxidants like Fe^{2+}/ascorbate. The possibility of a sample to slow the formation of peroxidative degradation products in such systems is an indication of its antioxidative capacity. Table 3 and Figure 2 show the results of the accumulation of TBARS (MDA) after different times (0, 30 min) of incubation of muscle homogenates. The differences between control (0.18 mg Se supplementation) and additionally Se supplemented animals (Sel group, 0.3 mg Se supplementation) are significant ($P < 0.05$) after 30 min incubation of homogenates of both MLLT and MSM. The differences in TBARS (MDA) content between control and Se supplemented animals are higher compared to earlier results (Krska *et al*., 2001; Nuernberg *et al*., 2002). The explanation could be in higher supplementation of Se level (basal 0.18 mg and additional 0.3 mg Se) in experimental pigs. Of course, supplementation with vitamin E to finishing pigs is more effective (Krska *et al*., 2001; Lahucky *et al*., 2001; Nuernberg *et al*., 2002) but together with Se the antioxidative defence system in carcasses of pigs can be improved.

The effects of Se supplementation on chemical composition and meat quality values are given in

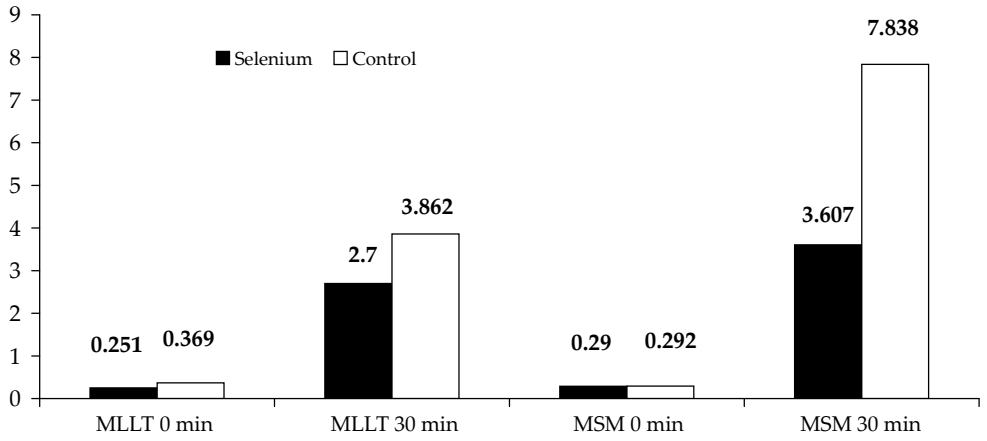

Figure 2. Comparison of antioxidative stability (MDA) in MLLT and MSM of the individual groups with selenium supplementation (μg/g)

Table 3. Effects of dietary organic selenium supplementation on the antioxidative capacity (malondialdehyde-MDA concentration, mg/g) of *m. longissimus lumborum et thoracis* (MLLT) and *m. semimembranosus* (MSM) in pigs (incubation of muscle homogenate with Fe^{2+}/ascorbate)

Trait	Group C		Group Sel	
	mean	± SEM	mean	± SEM
m. longissimus lumborum et *thoracis*				
incubation 0 min	0.369	0.018	0.251[a]	0.040
30 min	3.862	0.183	2.700[a]	0.250
m. semimembranosus				
incubation 0 min	0.292	0.009	0.290	0.010
30 min	7.838	1.059	3.607[a]	0.415

Group C = control group; Group Sel = group with organic selenium
[a]$P < 0.05$

Table 4. Chemical composition and meat quality (*m. longissimus lumborum et thoracis*) of control (group C) and supplemented (group Sel) pigs

Trait	Group C		Group Sel	
	mean	± SEM	mean	± SEM
Total protein (%)	24.44	0.19	24.68	0.26
Intramuscular fat (%)	2.65	0.21	2.38	0.12
pH 45 min	6.28	0.06	6.35	0.09
pH 24 h	5.86	0.04	5.90	0.05
Colour (reflectance %) 24 h	25.06	1.24	26.10	1.11
Conductivity (μS) 24 h	5.31	0.41	4.97	0.37
Drip loss (%) 48 h	6.98	0.51	6.26	0.44
MFI 5 days	90.02	11.94	101.91	5.52

MFI = myofibrillar fragmentation index (relative units)

Table 4. Dietary Se supplementation did not influence the total protein level in MLLT. There was a tendency of lowering intramuscular fat percentage in pigs supplemented with additional selenium ($P > 0.05$). The results of Mahan *et al*. (1999) and ours indicated that the *m. longissimus lumborum et thoracis* pH (45 min, 24 h) did not seem to be affected by the dietary Se levels and only a tendency of higher pH (45 min) was observed in our experiment ($P > 0.05$). The differences in colour (reflectance %) and conductivity values measured 24 h *post mortem* were also insignificant ($P > 0.05$), which is in agreement with the results reported by Krska *et al*. (2001) where colour stability (MLLT, 7 day storage) was unaffected by the administration of higher levels of vitamin E and Se. Drip loss of MLLT during the 48 h measurement period tended to be lower when the higher level Se was fed. Mahan *et al*. (1999) reported that the drip loss from the loin tissue of pigs receiving additional Se (0.3 mg/kg) was similar to that of the pigs fed on the basal diet (0.1 mg Se/kg), but pigs fed on inorganic Se tended to have a higher water loss from the loin tissue than those fed on the organic Se source. Recently Downs *et al*. (2000) showed the low drip loss of chicken breast fillets (*m. pectoralis major*) reduced approximately by 17% when organic selenium replaced sodium selenite in broiler diets to supply between 0.1 and 0.3 ppm Se. Spallholz (1994) reported in a review that selenite was more toxic in animal tissue than selenomethionine, and that the inorganic form could possess prooxidant properties. The differences in myofibrillar fragmentation index (MFI) were not significant ($P > 0.05$) and the results did not indicate a high influence on muscle aging (5 days) by the higher level of dietary Se. The discrepancy found in the literature regarding the meat quality values (mainly drip loss, pH 45 min) could also be a consequence of different levels of Se administration and/or the unknown halothane (malignant hyperthermia) gene status of pigs in some studies. In our studies experimental pigs were defined on the MH gene status and equal numbers of homozygotes and heterozygotes were used in the experiment.

The results showed that additional administration of organically bound Se (0.3 mg Se/kg feed for 97 days) to basal diet (0.18 mg Se/kg) significantly ($P < 0.05$) improved the amount of Se and antioxidative capacity in muscle tissues of pigs and positively influenced meat quality values to some extent.

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ABSTRAKT

Vplyv prídavku organického selénu v krmive na obsah selénu, antioxidačnú kapacitu svalov a kvalitu mäsa ošípaných

Cieľom práce bolo zistiť vplyv prídavku organického selénu v krmive na obsah selénu a antioxidačnú kapacitu svalu (*m. longissimus lumborum et thoracis* – MLLT, *m. semimebranosus* – MSM) a kvalitu mäsa ošípaných definovaných na výskyt malígnej hypertermie (DNA test). Aplikácia prídavku organického selénu (0,3 mg Se/kg KZ) k štandardnej kŕmnej zmesi (s obsahom 0,18 mg Se/kg KZ) sa robila 97 dní pred zabitím. Po zabití (v priemere 105 kg) sa vo svale MLLT stanovili hodnoty pH (45 min, 24 h), farby a elektrickej vodivosti (24 h), straty odkvapom (48 h) a myofibri-

lárny fragmentačný index (5 dní). Ďalej sa vo svale MLLT stanovil obsah celkových bielkovín a intramuskulárneho tuku (Infratec). Antioxidačná kapacita (obsah látok typu malondialdehydu, MDA, po reakcii s kyselinou thiobarbiturovou) sa zisťovala z homogenátu svalu (MLLT, MSM) po stimulovaní oxidácie inkubáciou (30 min) v zmesi Fe^{2+}/askorbát. Obsah selénu vo vzorkách svalov (MLLT, MSM) sa zistil spektrofluorometrickou metódou. Zistili sme významne vyšší ($P < 0,05$) obsah selénu (0,377 vs. 0,922 mg/kg – MLLT and 0,377 vs. 0,836 mg/kg – MSM) u ošípaných kŕmených so zvýšeným prídavkom organického selénu. Významné zníženie ($P < 0,05$) obsahu MDA vo svaloch MLLT a MSM poukázalo na zvýšenie antioxidačnej kapacity svalu. Zistili sme tendencie nižších hodnôt strát odkvapom, ale rozdiely medzi skupinami ošípaných boli pod hranicou významnosti ($P > 0,05$). Aplikácia zvýšeného prídavku organického selénu (0,3 mg Se/kg KZ) do krmiva (s obsahom 0,18 mg Se/kg KZ) 97 dní pred zabitím má významný vplyv ($P < 0,05$) na zvýšenie obsahu selénu a zvýšenie antioxidačnej kapacity vo svaloch (MLLT, MSM) ošípaných.

Kľúčové slová: ošípané; selén; antioxidačný stav; kvalita mäsa

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