Effects of high dietary levels of Se-enriched yeast on tissues selenium content and meat quality traits – a model study in rats

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Key words: selenomethionine; sodium selenite; lipid oxidation; glutathione peroxidase; pig

Abstract

OBJECTIVES: The aim of the trial was to evaluate effects of high dietary levels of Se-enriched yeast on selenium concentrations in muscle and liver tissue and on the meat quality traits in rats.

DESIGN: The experimental feed mixtures were fed to Wistar Albino rats for a period of 10 weeks. They differed in the dose and in the form of Se supplement. Supplemental dose of 0.3 mg.kg⁻¹ of Se per one kilogram of feed was used in group I (n = 10, sodium selenite) and in group II (n = 10, Se-enriched yeast). Elevated dose 2 mg.kg⁻¹ Se per one kilogram of feed was used in group III (n = 10, sodium selenite) and in group IV (n = 10, Se-enriched yeast).

RESULTS: The use of elevated dietary Se level (2 mg.kg⁻¹) in the form of sodium selenite did not increase lipid oxidation (malondialdehyde content) in muscles nor influenced meat quality traits. The use of elevated dietary Se level in the form of Se-enriched yeast resulted in higher Se muscle concentrations accompanied by increased malondialdehyde content. The meat quality traits remained unaffected.

CONCLUSION: High selenium concentrations in muscle produced by Se-enriched yeast were accompanied by elevated MDA levels, but it did not negatively influence meat quality traits.

Abbreviations:

ALP - alanine aminophosphatase
ALT - alanine aminotransferase
AST - aspartate aminotransferase
CK - creatine kinase
Ca - calcium
GSH-Px - glutathione peroxidase
HCl - hydrochlorid acid
LDH - lactate dehydrogenase
MDA - malondialdehyde
P - phosphorus
SeMet - selenomethionine
TBARS - thiobarbituric acid reactive substance
TG - triglycerides
TP - total protein
w.w. - wet weight
INTRODUCTION

Selenium is an important trace element. It is incorporated as selenocysteine at the active site of various proteins such as glutathione peroxidase (Tappel 1974) and thioredoxin reductases (Brown & Arthur 2001) and it protects organism against oxidative stress (Bujalska & Gumulka 2008). Adequate selenium intake is essential for human health. Its deficiency has been associated with number of health disorders such as Keshan disease (Finley 2006), cardiovascular disease (Brown & Arthur 2001) and cancer (Combs 1999).

As pork is consumed in large quantities throughout the world, it would be a convenient Se source for human diet. The results of our study show that pork contributes significantly to the selenium intake of the human population in the Czech Republic (Fajt et al. 2009). And the results of our other study support the hypothesis that there may be breed differences in indices of selenium status in pigs (Banoch et al. 2009). Inorganic sodium selenite and organic Se-enriched yeast are used in finisher pig diets. The Se-enriched yeast contains selenium mainly as selenomethionine (Schrauzer 2000) and provides higher concentrations of Se in muscle tissue than inorganic Se form (Whanger & Butler 1988; Mahan & Parrett 1996). The high selenium retention in tissues is caused by the fact that non-metabolized selenomethionine is incorporated non-specifically into body proteins in place of methionine (Schrauzer 2000).

This raised concerns that Se from selenomethionine could build up in body tissues to toxic levels (Rayman 2000). The maximum dose 0.3 mg.kg⁻¹ of Se per one kilogram of feed has been used so far in finisher swine studies. The aim of the study was to find out whether employments of higher Se-enriched yeast doses could affect negatively biochemical plasma indices including of GSH-Px, selenium concentration in striated muscle and liver, lipid oxidation (MDA) in tissues and meat quality. We decided to conduct a model study on rats.

MATERIAL AND METHODS

Altogether 40 female weanling Wistar Albino rats were used in the study. The rats were fed experimental feed mixtures for a period of 10 weeks. The feed mixtures were composed from a basal diet and a mineral supplement. The composition of the basal diet is presented in Table 1.

The experimental animals were divided into 4 groups that differed in the form of the mineral supplement and in the dose. The animals were fed and watered ad libitum. They were kept in room with 12 hours change of light and dark and with continual temperature of 22 ± 2°C.

In group I (n = 10) the feed was supplemented with 0.3 mg.kg⁻¹ of Se per one kilogram of feed in the form of sodium selenite.

In group II (n = 10) the Se-enriched yeast (SelPlex) was used as a selenium supplement at the dose 0.3 mg.kg⁻¹ of Se.

Elevated selenium doses were used in groups III and IV. The rats in group III (n = 10) received feed mixture supplemented with 2 mg.kg⁻¹ of Se as sodium selenite.

In group IV (n = 10) the feed mixture was supplemented with 2 mg.kg⁻¹ of Se in the form of Se-enriched yeast (SelPlex).

Sampling

After 10 weeks the rats were anesthetized i.m. with xylazin 2% inj. and ketamin 1% inj. and blood was taken by heart puncture. Heparin was used as anticoagulant for biochemical analysis in blood plasma. The rats were then euthanized by cervical dislocation and the tissue samples were collected (striated muscle, liver).

Biochemical analysis in blood plasma

The remaining heparinized blood was centrifuged (800 g, 10 min, 4°C) to separate plasma. Assessed plasma biochemical indices (albumin, TP, ALP, ALT, AST, cholesterol, creatine kinase, creatinine, phosphorus, calcium, LDH, lactate, urea and TG) were measured with the biochemical analyzer Cobas EMira using commercial test kits (BioVendor, Czech Republic). GSH-Px activities were determined according to the method of Paglia and Valentin (1967) using the test kit RANSEL (Randox Laboratories, Ltd., United Kingdom).

Muscle and liver analysis

The samples for meat measurements were taken from rats’ leg (musculus biceps femoris). Selenium concentration in tissues (striated muscle, liver) were determined by the Solar 939 AA spectrometer (Unicam, United Kingdom) using a hydride AAS technique.

The samples were prepared by mineralization in a closed system using a microwave (MLS-1200, Milestone, Italy) digestion technique with HNO₃ and H₂O₂. After the mineralization the samples were evaporated and the mineral residue dissolved in water to which 20% HCl was added.

Tab 1. The composition of basal diet.

<table>
<thead>
<tr>
<th>Parametre [units]</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein [g.kg⁻¹]</td>
<td>212</td>
</tr>
<tr>
<td>Crude fat [g.kg⁻¹]</td>
<td>38</td>
</tr>
<tr>
<td>Crude fiber [g.kg⁻¹]</td>
<td>44</td>
</tr>
<tr>
<td>Crude ash [g.kg⁻¹]</td>
<td>67</td>
</tr>
<tr>
<td>Starch [g.kg⁻¹]</td>
<td>350</td>
</tr>
<tr>
<td>Sugar [g.kg⁻¹]</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin A [IE/IU]</td>
<td>15 000</td>
</tr>
<tr>
<td>Vitamin D₃ [IE/IU]</td>
<td>1 000</td>
</tr>
<tr>
<td>Vitamin K₂ [mg.kg⁻¹]</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin K₃ [mg.kg⁻¹]</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin C [mg.kg⁻¹]</td>
<td>0</td>
</tr>
<tr>
<td>Copper [mg.kg⁻¹]</td>
<td>5</td>
</tr>
</tbody>
</table>
The surface colour of raw rats meat was measured by the CIE L*a*b* system using Minolta CM 2600d (Konica Minolta, Japan). Measuring area of 3 mm, illuminant D65 and 10° standard observer were used. The instrument was standardized using a standard white plate. CIE L* – lightness, a* – redness, b* – yellowness, Chroma C* = (a*^2 + b*^2)^0.5 and hue h° = tan^(-1)(b*/a*). The result stated for each sample is the mean value of five measurements. Colour was measured 1 hour and 24 hours after euthanasia.

Drip loss was determined as a percentage of weight loss after 24 hours storage at 4 °C.

pH value (1 h and 24 h after euthanasia) was measured with a needle probe SenTix Sp on pH-meter 340i WTW (WTW, Germany).

A degree of lipid oxidation was measured by reaction with thiobarbituric acid after distillation – TBARS value (Thiobarbituric acid reactive substance) (Castellini et al. 2002). Aliquots of sample (5 g) were transferred to a flat-bottomed flask and one drop of silicone anti-foaming agent added plus 2.5 ml HCl and 97.5 ml distilled water. This sample was then distilled and the first 50 ml of distillate collected. Next 5 ml of the distillate were added to 5 ml 0.02 M thiobarbituric acid and heated in a boiling water bath for 35 minutes for color development. Malondialdehyde (MDA) was distilled in duplicate from the sample and its absorbance was determined at 532 nm in a 1 cm glass cell. MDA was measured in muscle and liver.

**Statistical analysis**

Statistical analysis of the data was performed using the program Statistica 8.0. for Windows (StatSoft, Ing. USA). Data were tested for normal distribution using Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) was applied to the differences in measured indices among tested groups. Individual differences among the means were tested using Tukey-HSD test and p<0.05 was chosen as the level of significance.

**RESULTS**

**Biochemical analysis**

No significant differences were observed in established biochemical indices. The results are presented in Table 2.

**Selenium concentration in tissues**

**Striated muscle**

At lower supplemental dose (0.3 mg.kg⁻¹ of Se) the Se muscle content of Se-yeast group (II) was not significantly higher than of sodium selenite group (I) (p=0.1333). At elevated dietary Se level (2 mg.kg⁻¹ of Se) the Se muscle concentrations were significantly higher in Se-yeast group (IV) (p=0.0001). In case of both Se forms the Se muscle content increased with higher supplemental dose. This increase was more pronounced in Se-yeast group (IV) (p=0.0001). There was also significant difference between concentrations 0.3 mg.kg⁻¹ of Se (II) and 2 mg.kg⁻¹ of Se in groups with SelPlex (IV) (p=0.0001). In groups with sodium selenite (I and III) we have found significant difference (p=0.0011). The results are presented in Figure 1.

**Liver**

The Se concentrations in liver were significantly higher (p=0.0001) in Se-yeast group at dietary level of 0.3 mg.kg⁻¹ of Se (II) than in selenite group (I). At dietary

<table>
<thead>
<tr>
<th>Indices [units]</th>
<th>Group I (mean±SD)</th>
<th>Group II (mean±SD)</th>
<th>Group III (mean±SD)</th>
<th>Group IV (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin [g.l⁻¹]</td>
<td>31.69±3.72</td>
<td>32.00±3.12</td>
<td>31.30±3.08</td>
<td>30.39±6.08</td>
</tr>
<tr>
<td>TP [g.l⁻¹]</td>
<td>57.34±3.91</td>
<td>56.41±4.89</td>
<td>57.84±3.81</td>
<td>55.57±5.07</td>
</tr>
<tr>
<td>ALP [μkat.l⁻¹]</td>
<td>1.74±0.28</td>
<td>1.96±0.37</td>
<td>1.43±0.15</td>
<td>1.94±0.33</td>
</tr>
<tr>
<td>ALT [μkat.l⁻¹]</td>
<td>0.84±0.19</td>
<td>0.80±0.12</td>
<td>0.75±0.12</td>
<td>1.12±0.45</td>
</tr>
<tr>
<td>AST [μkat.l⁻¹]</td>
<td>1.73±0.54</td>
<td>1.92±0.98</td>
<td>1.62±0.42</td>
<td>3.34±2.35</td>
</tr>
<tr>
<td>Cholesterol [mmol.l⁻¹]</td>
<td>2.36±0.39</td>
<td>2.33±0.31</td>
<td>2.18±0.21</td>
<td>2.03±0.31</td>
</tr>
<tr>
<td>CK [μkat.l⁻¹]</td>
<td>11.62±10.08</td>
<td>16.16±10.04</td>
<td>7.90±5.14</td>
<td>12.48±6.32</td>
</tr>
<tr>
<td>Creatinine [μmol.l⁻¹]</td>
<td>51.96±5.92</td>
<td>53.27±5.56</td>
<td>50.03±3.70</td>
<td>53.19±7.95</td>
</tr>
<tr>
<td>P [mmol.l⁻¹]</td>
<td>1.50±0.52</td>
<td>1.62±0.22</td>
<td>1.87±0.23</td>
<td>2.86±1.08</td>
</tr>
<tr>
<td>Ca [mmol.l⁻¹]</td>
<td>2.46±0.10</td>
<td>2.41±0.13</td>
<td>2.46±0.06</td>
<td>2.54±0.09</td>
</tr>
<tr>
<td>LDH [μkat.l⁻¹]</td>
<td>5.16±2.92</td>
<td>5.13±2.45</td>
<td>5.85±4.04</td>
<td>4.70±1.62</td>
</tr>
<tr>
<td>Lactate [mmol.l⁻¹]</td>
<td>5.00±2.19</td>
<td>4.31±0.69</td>
<td>4.34±1.18</td>
<td>3.28±1.33</td>
</tr>
<tr>
<td>Urea [mmol.l⁻¹]</td>
<td>6.53±0.56</td>
<td>5.92±0.50</td>
<td>6.11±0.38</td>
<td>6.33±0.59</td>
</tr>
<tr>
<td>TG [mmol.l⁻¹]</td>
<td>1.11±0.36</td>
<td>1.05±0.38</td>
<td>1.33±0.29</td>
<td>0.71±0.22</td>
</tr>
<tr>
<td>GSH-Px TG[μkat.l⁻¹]</td>
<td>398.00±48.20</td>
<td>400.00±10.40</td>
<td>385.00±56.33</td>
<td>405.00±80.43</td>
</tr>
</tbody>
</table>
level of 2 mg.kg⁻¹ of Se the Se concentrations in liver were comparable between the two forms (p=0.7886). When Se-yeast was used the selenium liver content did not increase with higher supplemental dose (II, IV) (p=0.1183). In group with sodium selenite there was a significant difference (p=0.0001) between groups (I, III). The results are presented in Figure 2.

**Lipid oxidation – malondialdehyde content in tissues**

**Striated muscle**
In concentrations of 0.3 mg.kg⁻¹ of Se there was no statistical significance between groups (p=0.2473). At the elevated dietary level (2 mg.kg⁻¹ of Se), the MDA muscle content was higher in Se-yeast group (IV) (p=0.002). The results are presented in Figure 3.

**Liver**
The amount of MDA in liver was the highest in Se-yeast group (0.3 mg.kg⁻¹) (II). We have found statistical differences between groups II and I (p=0.0005), between groups II and IV (p=0.001). At the dose 2 mg.kg⁻¹ of Se the MDA content was no significant difference between groups III and IV (p=0.1045). The results are presented in Figure 4.

**Meat quality traits**
The results of meat quality traits are given in Table 3. At lower supplemental level no differences in pH, colour characteristics and drip loss were found between the two Se forms.

The increase of dietary Se level did not produce significant changes in meat quality. Also at the elevated dietary Se level no statistically significant differences in meat quality traits were found between the two Se forms.

**DISCUSSION**
The Wistar Albino rat is a suitable model for monogastric animal studies. Moreover the dose 0.3 mg.kg⁻¹ of Se per one kilogram of feed used in finisher swine is also recommended for rats (Behne & Wolters 1983; Whanger & Butler 1988). The dose 2 mg.kg⁻¹ of Se per one kilogram of feed was significantly elevated, but far from being toxic. This was confirmed by the fact that neither biochemical examination indicated any toxic effects of the elevated dose. In fact toxicity studies on rats revealed that LD₅₀ for Se-enriched yeast is 37.3 mg.kg⁻¹ and for sodium selenite 12.7 mg.kg⁻¹ (Vinson & Bose 1987).

The Se-enriched yeast at the dose 0.3 mg.kg⁻¹ of Se produced statistically higher Se concentrations in liver. When dose 2 mg.kg⁻¹ of Se per one kilogram of feed was used much more pronounced differences between Se-enriched yeast and sodium selenite were found in muscle tissue. This is in agreement with results of Whanger (1986) and it indicates that the magnitude of difference in muscle tissue increases at higher dietary Se levels. This can be explained by the existence of tissue threshold for sodium selenite. Once the physiological stores are filled, the body excretes any excess of Se in urine and breathe (Lindberg & Lannek 1965; Rayman 2000).

We assume that at elevated dietary level (2 mg.kg⁻¹ of Se) the selenium muscle content produced by sodium selenite had already reached its plateau. In case of Se-yeast, the excess of SeMet is incorporated nonspecifically into body proteins (Schrauzer 2000).

This allows its accumulation in tissues. Interestingly the elevation of dietary Se level did not result in higher

<table>
<thead>
<tr>
<th>Indices</th>
<th>Group I (mean ±SD)</th>
<th>Group II (mean ±SD)</th>
<th>Group III (mean ±SD)</th>
<th>Group IV (mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*1</td>
<td>45.30±2.43</td>
<td>46.54±2.69</td>
<td>46.23±2.16</td>
<td>45.81±4.24</td>
</tr>
<tr>
<td>L*24</td>
<td>45.57±2.16</td>
<td>48.10±2.21</td>
<td>47.56±2.41</td>
<td>46.04±3.82</td>
</tr>
<tr>
<td>a*1</td>
<td>5.22±1.10</td>
<td>3.50±2.41</td>
<td>2.35±1.08</td>
<td>3.39±1.99</td>
</tr>
<tr>
<td>a*24</td>
<td>2.29±0.84</td>
<td>1.67±2.08</td>
<td>1.59±0.94</td>
<td>1.86±1.02</td>
</tr>
<tr>
<td>b*1</td>
<td>7.99±1.06</td>
<td>6.80±1.99</td>
<td>5.59±1.66</td>
<td>6.99±1.40</td>
</tr>
<tr>
<td>b*24</td>
<td>4.69±0.93</td>
<td>4.91±1.50</td>
<td>3.66±1.09</td>
<td>4.83±0.66</td>
</tr>
<tr>
<td>C*1</td>
<td>10.30±2.41</td>
<td>7.94±2.51</td>
<td>6.24±1.71</td>
<td>8.08±1.71</td>
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<tr>
<td>C*24</td>
<td>5.33±0.84</td>
<td>5.42±2.16</td>
<td>4.13±1.21</td>
<td>5.67±1.24</td>
</tr>
<tr>
<td>h*1</td>
<td>56.59±4.64</td>
<td>64.61±9.10</td>
<td>66.94±9.63</td>
<td>66.26±9.07</td>
</tr>
<tr>
<td>h*24</td>
<td>63.46±8.95</td>
<td>76.30±6.64</td>
<td>73.87±6.56</td>
<td>68.02±7.01</td>
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<tr>
<td>pH 1</td>
<td>6.38±0.24</td>
<td>6.06±0.10</td>
<td>6.23±0.15</td>
<td>6.28±0.20</td>
</tr>
<tr>
<td>pH 24</td>
<td>6.08±0.10</td>
<td>6.03±0.09</td>
<td>6.06±0.10</td>
<td>6.07±0.08</td>
</tr>
<tr>
<td>Drip loss [%]</td>
<td>3.26±0.75</td>
<td>3.61±1.06</td>
<td>3.14±1.18</td>
<td>2.88±0.55</td>
</tr>
</tbody>
</table>

L* – lightness, a* – redness, b* – yellowness, C* – Chroma, h* – hue
Se liver content in Se-yeast group. This indicates that the liver has limited capacity to accumulate SeMet, the excess being accumulated in other tissues.

Se-enriched yeast and sodium selenite did not differ in plasma GSH-Px activities. This is in line with Brown et al. (2000) and Mahan and Parrett (1996) and confirms that both Se forms are adequate for GSH-Px synthesis. The increase of dietary Se levels was not accompanied by changes in GSH-Px activities. Therefore we assume that plasma GSH-Px activities reached its plateau already at the first dietary level (0.3 mg.kg–1 of Se).

The accumulation of Se in muscle tissue produced by high dietary Se-yeast (2 mg.kg–1 of Se) resulted in increased lipid oxidation (higher MDA content in muscles). To explain this finding following mechanisms should be considered. SeMet is incorporated non-specifically into body proteins (Schrauzer 2000). Alternatively it can be trans-selenated to selenocysteine. Selenocysteine is then converted to hydrogen selenite (Rayman 2000). Hydrogen selenite is able to generate reactive oxygen species by oxidizing thiols and reducing oxygen to superoxid (Xu et al. 1991). Under normal conditions the excess of hydrogen selenite is detoxified by successive methylation and excreted in breath and urine (Rayman 2000). It is possible that an excessive retention of SeMet in tissues increases the amount of non-detoxified hydrogen selenite and thus generates the oxidative stress.

Sodium selenite is a compound with prooxidative potential (Spallholz 1994) and according to Mahan and Parrett (1996) it might affect negatively meat quality. This is contradictory to our results. The use of high dietary level of sodium selenite did result neither in increased lipid oxidation nor in negative changes of meat quality. This can be explained by the fact that Se from sodium selenite builds up in tissues only to physiological levels any excess being excreted (Lindberg & Lannek 1965).

The use of Se-yeast (2 mg.kg–1 of Se) resulted in higher lipid oxidation (higher MDA content) in muscle than in sodium selenite group. Although no statistically significant differences in meat quality traits were found.

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**Fig. 1.** Se concentration in muscle (mean±SD), significant differences (p<0.05) are indicated by different alphabetic superscript.
I-0.3 mg.kg–1 of sodium selenite, II-0.3 mg.kg–1 of SelPlex, III-2.0 mg.kg–1 of sodium selenite, IV-2.0 mg.kg–1 of SelPlex

**Fig. 2.** Se concentration in liver (mean±SD), significant differences (p<0.05) are indicated by different alphabetic superscript.
I-0.3 mg.kg–1 of sodium selenite, II-0.3 mg.kg–1 of SelPlex, III-2.0 mg.kg–1 of sodium selenite, IV-2.0 mg.kg–1 of SelPlex

**Fig. 3.** MDA in muscle (mean±SD), significant differences (p<0.05) are indicated by different alphabetic superscript.
I-0.3 mg.kg–1 of sodium selenite, II-0.3 mg.kg–1 of SelPlex, III-2.0 mg.kg–1 of sodium selenite, IV-2.0 mg.kg–1 of SelPlex

**Fig. 4.** MDA in liver (mean±SD), significant differences (p<0.05) are indicated by different alphabetic superscript.
I-0.3 mg.kg–1 of sodium selenite, II-0.3 mg.kg–1 of SelPlex, III-2.0 mg.kg–1 of sodium selenite, IV-2.0 mg.kg–1 of SelPlex
between the two groups. It is known that the oxidative stress can lead to increased drip loss (impairment of muscle cell membrane integrity) and paler meat color (oxidation of oxymyoglobin to metmyoglobin) (Zhan et al. 2007; Schaefer et al. 1995).

We suggest that in case of our study the MDA content did not differ to such an extent to result in statistically significant differences in meat quality.

Moreover the MDA content is not a direct indicator of oxymyoglobin oxidation. The low pH is considered as a more important factor affecting drip loss than the oxidative stress (den Hertog-Meischke et al. 1997). In our study muscle pH values did not differ between the two sources.

It can be concluded that high selenium concentrations in muscle produced by Se-enriched yeast were accompanied by elevated MDA levels, but it did not negatively influence meat quality traits.

ACKNOWLEDGEMENT

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