

1  
2  
3

# JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Exploring effects of organic selenium supplementation on pork loin: Se content, meat quality, antioxidant capacity, and metabolomic profiling during Storage
Running Title (within 10 words)	Effect of organic selenium on pork quality and antioxidant capacity
Author	Hyun Young Jung <sup>1</sup> , Hyun Jung Lee <sup>2</sup> , Hag Ju Lee <sup>1</sup> , Yoo Yong Kim <sup>1,3</sup> , Cheorun Jo <sup>1,2,3,*</sup>
Affiliation	<sup>1</sup> Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea <sup>2</sup> Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Korea <sup>3</sup> Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang
ORCID (for more information, please visit <a href="https://orcid.org">https://orcid.org</a> )	Hyun Young Jung ( <a href="https://orcid.org/0000-0002-4561-9304">https://orcid.org/0000-0002-4561-9304</a> ) Hyun Jung Lee ( <a href="https://orcid.org/0000-0002-6891-8008">https://orcid.org/0000-0002-6891-8008</a> ) Hag Ju Lee ( <a href="https://orcid.org/0000-0003-2906-7666">https://orcid.org/0000-0003-2906-7666</a> ) Yoo Yong Kim ( <a href="https://orcid.org/0000-0001-8121-3291">https://orcid.org/0000-0001-8121-3291</a> ) Cheorun Jo ( <a href="https://orcid.org/0000-0003-2109-3798">https://orcid.org/0000-0003-2109-3798</a> )
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This research was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the Useful Agricultural Life Resources Industry Technology Development Project, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (120051022SB010).
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Lee Hyun Jung, Kim YY, Jo C Data curation: Lee Hyun Jung Formal analysis: Jung HY, Lee Hag Ju Methodology: Jung HY, Lee Hyun Jung Software: Validation: Investigation: Jung HY, Lee Hag Ju Writing - original draft: Jung HY, Lee Hyun Jung Writing - review & editing: Jung HY, Lee Hyun Jung, Lee Hag Ju, Kim YY, Jo C
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

4  
5

## CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Cheorun Jo
Email address – this is where your proofs will be sent	cheorun@snu.ac.kr

Secondary Email address	
Address	Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea
Cell phone number	+82-10-3727-6923
Office phone number	+82-2-880-4804
Fax number	+82-2-880-2271

6  
7

8     **Abstract**

9     This research was conducted to study the effects of organic selenium (Se) supplements at different levels  
10    on pork loin quality during storage. Fifteen pork loins were procured randomly from three groups, Con  
11    (fed basal diet), Se15 (fed 0.15 ppm organic Se along with 0.10 ppm inorganic Se), and Se45 (fed 0.45  
12    ppm organic Se along with 0.10 ppm inorganic Se). Each sample was analyzed for Se contents,  
13    antioxidant properties [glutathione peroxidase activity, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic  
14    acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, 2-thiobarbituric  
15    acid reactive substances], physicochemical properties (water holding capacity, pH, color), and  
16    metabolomic analysis during 14-day storage period. Se45-supplemented group showed significantly  
17    higher Se contents and glutathione peroxidase activity than the other groups throughout the storage period.  
18    However, other antioxidant properties were not significantly affected by Se supplementation. Selenium  
19    supplementation did not have an adverse impact on physicochemical properties. NMR-based  
20    metabolomic analysis indicated that the selenium supply conditions were insufficient to induce metabolic  
21    change. These results suggest that organic Se (0.15 and 0.45 ppm) can accumulate high Se content in pork  
22    loins without compromising quality.

23

24    **Keywords:** Pork loin, Selenium supplementation, Meat quality, Antioxidant properties, Metabolites

25

26

## Introduction

Pork feed primarily consists of soybean meal and corn, supplemented with various additives such as vitamins and minerals to control the growth rate of pigs [1, 2]. The composition of pig feed can also influence pork quality [3]. Many studies have been conducted to improve both pork production and quality by supplementing pig feed with various additives, including antioxidants [4]. Vitamin C, vitamin E, and selenium (Se) have been used as antioxidants in feed, and previous research has shown that their use can modulate the antioxidant capacity, nutritional quality, and fatty acid composition of pork [1, 5].

Se is commonly used in pork farming due to its regulatory and immune system function [6, 7]. It can also improve pork quality and nutritional value as it is an essential component of glutathione peroxidase (GPx) [8, 9]. GPx is one of the antioxidant enzymes that can reduce lipid hydroperoxides and free hydrogen peroxide in body tissues [10]. Therefore, Se supplementation can increase GPx activity, potentially improving antioxidant capacity of pork [11].

Se exists in two chemical forms in nature, organic and inorganic [12]. Inorganic Se, mainly in the form of selenite and selenium salts, is commonly used in pork feed due to its easy supply and cost-effectiveness [13]. However, the use of inorganic Se has limitations such as low accumulation rate in the body despite high digestion and absorption rate [14], lower absorption rate compared to organic Se [15], and potential toxic effects at high levels [16].

On the other hand, organic Se, in the form of selenomethionine and selenium-yeast, has a higher accumulation efficiency and antioxidant activity when fed to livestock [17, 18]. It can also prevent Se deficiency, which frequently occurs in weaning piglets when fed to sows [19]. In addition, organic Se has been reported to delay the post-oxidative reaction of the muscle, improving the nutritional value, flavor, and shelf life of meat, as well as meat color and water holding capacity [20, 21, 22]. Despite being expensive, organic Se has been considered for pig feeding [23].

Recently, there has been emphasis on converting feed supplements from inorganic Se to organic Se due to the limitation of Se and the potential benefits of organic Se [24]. However, economic feasibility is an important factor in livestock industry, and the conversion rate must be considered. Several studies are currently underway to replace and/or combine inorganic Se with organic Se, and some have reported improved antioxidant performance and health levels [25]. While we have confirmed the combined effect of inorganic and organic Se on the growth performance of pigs at different levels (data not shown), their effect on antioxidant capacity and quality has not been studied for our market consumers. Therefore, we evaluated the combined effect of inorganic and organic Se on the quality of pork loin during refrigerated storage.

## Materials and Methods

## 62 **Sample preparation**

63

64 A total of 105 growing pigs [(Yorkshire × Landrace) × Duroc] with an average body weight of  $39.85 \pm$   
65  $0.01$ kg were divided into 15 pens with 7 pigs in a randomized complete block design. The pigs were kept  
66 in climate-controlled facility that had a fully concrete floor measuring 2.4 by 2.9 m<sup>2</sup>. A feeder and a  
67 nipple drinker were provided in each pen to ensure that the pigs had unrestricted access to food and water.  
68 The experimental period was 14 weeks during with three types of experimental treatments were  
69 implemented. Each of the 5 pens was assigned to one of 3 treatment groups, resulting 5 pens per group.  
70 The experimental treatments were as follows: Con (fed basal diet), Se15 (fed 0.15 ppm organic Se along  
71 with 0.10 ppm inorganic Se), and Se45 (fed 0.45 ppm organic Se along with 0.10 ppm inorganic Se).  
72 Each treatment group was fed with 0.10 ppm of inorganic Se (Genebiotech, Gongju, Korea), while the  
73 addition of organic Se (Sel-Plex™, Alltechm Inc., Nicholasville, USA) was adjusted to induce Se  
74 accumulation in pork. The transformation from inorganic to organic Se was accomplished by partially  
75 modifying the feeding quantity of inorganic Se. From each group, 5 pigs were randomly selected and their  
76 loins (*M. longissimus*) were obtained. The samples were cut into 3 pieces ( $330 \pm 20$  g) and packaged in  
77 air permeable bags. They were then stored at 4°C, and the following experiments were conducted on days  
78 0, 7 and 14. On each storage day, water holding capacity (WHC), pH, and meat color were analyzed  
79 immediately, and the samples were frozen at  $-70$  °C until further analyses.

80

## 81 **Se content**

82

83 The Se concentration in pork loins was determined using the fluorometric method of AOAC (2000)  
84 [22]. To perform the analysis, 0.5 g of the sample was added to a screw cap culture tube containing 5 mL  
85 of a mixed solution of HClO<sub>4</sub> (perchloric acid 70%) and HNO<sub>3</sub> (nitric acid 70%) in 1:4 ratio. The culture  
86 tube was digested for 4 hours in a digestion block at 210 °C, then cooled down in room temperature. After  
87 cooling, add 0.5 mL HCl was added to the tube and the tube was heated at 150 °C for 30 min. Then, the  
88 tube was cooled again, and 15 mL of 0.1M EDTA solution and 2 mL of 0.1 % 2,3-diaminonaphthalene  
89 solution were added. The tube was vortexed for 5 sec and incubate in a water bath at 60 °C for 30 min.  
90 Following incubation, a 10-second vortexing of the tube was done after adding 5 mL of cyclohexane. The  
91 extracted cyclohexane layer was transferred to a cuvette, and the absorbance was measured using 369 nm  
92 excitation and 525 nm emission settings.

93

## 94 **GPx activity**

95 The activity of GPx activity was measured through the utilization of Glutathione Peroxidase Assay Kit  
96 (353919, Sigma-Aldrich, Burlington, USA). Briefly, minced meat sample (5 g) was homogenized with 25  
97 mL of cold homogenization buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1 mM DTT) at 12,000 rpm

98 for 1 min (T25 digital ULTRA-TURRAX<sup>®</sup>, Ika Co., Staufen, Germany). The homogenized sample was  
99 centrifuged (Continent 512 R, Hanil Co., Ltd., Incheon, Korea) at 10,000×g for 15 min, and the  
100 supernatant was taken. The Assay Buffer, Co-Substrate Mixture, and NADPH included in the kit were  
101 mixed with the supernatant. Then, the reaction was initiated by adding hydroperoxide. Thereafter, the  
102 absorbance was measured at 340 nm every min for 10 min to confirm the GPx activity.

103

#### 104 **Antioxidant activity**

105 Ground sample (3 g) was homogenized with 12 mL of deionized distilled water at 9,600 rpm for 30 s  
106 (T25 digital ULTRA-TURRAX<sup>®</sup>, Ika Co.). The homogenized samples were centrifuged (Continent 512 R,  
107 Hanil Co., Ltd.) at 2,265×g for 10 min, and filtered using filter paper (No. 1, Whatman PLC., Maidstone,  
108 UK). For the meat extract, after centrifuging at 2,265×g for 10 min, 10 mL of chloroform was added to  
109 the filtrate.

110 For the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, a solution of 14 mM  
111 ABTS and 4.9 mM potassium persulfate was prepared and left in the dark for 16 minutes after vigorous  
112 vortexing. The subsequent steps were performed following the protocol described by Choe et al. [26].

113 For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 1 mL of the diluted meat extract was mixed with  
114 1 ml of 0.2 mM DPPH in methanol, vortexed, and placed in the dark for 30 min at room temperature. The  
115 subsequent steps were performed following the protocol described by Choe et al. [26].

116 For the 2-thiobarbituric acid reactive substances (TBARS) assay, the meat sample (5 g) was  
117 homogenized with 15 mL of deionized distilled water and 50 μL of 7.2% butylated hydroxy toluene  
118 solution at 9,600 rpm for 30 s (T25 digital ULTRA-TURRAX<sup>®</sup>, Ika Co.). Then, the subsequent steps  
119 were followed by Rupasinghe et al. [27]

120

#### 121 **Physicochemical analysis**

122

123 Minced meat sample (5 g) was placed on a filter paper and centrifuged at 252×g for 10 min (Continent  
124 512R, Hanil Co., Ltd.). The WHC was measured as described by Kwon et al. [28] and pH by Rupasinghe  
125 et al. [27], respectively. The meat color of pork loin was measured using a colorimeter (CM-5, Konica  
126 Minolta Co., Ltd., Osaka, Japan). Prior to measurement, the colorimeter was calibrated with a standard  
127 black plate. The meat color was measured at three different locations on the top and the bottom of each  
128 sample [22]. The color value was expressed as CIE L\*, a\*, b\* and delta E was calculated as  $\sqrt{(\Delta L^*)^2 +$   
129  $(\Delta a^*)^2 + (\Delta b^*)^2}$ .

130

#### 131 **Nuclear Magnetic Resonance (NMR)-based metabolic analysis**

132

133 The NMR analysis was performed according to Kim et al. (2021) [29]. In brief, each minced sample (5  
134 g) was homogenized with 20 mL of 0.6 M perchloric acid at 12,000 rpm for 1 min (T25 digital ULTRA-  
135 TURRAX®, IKA Co.). The homogenized samples were centrifuged at 2,265×g for 20 min (Continent  
136 512R, Hanil Co., Ltd.), and the supernatant was transferred in another test tube and adjusted to 7.0 with  
137 sodium hydroxide. Then, the subsequent steps were performed following the method [29]

138

### 139 **Statistical analysis**

140

141 The data were analyzed using two-way analysis of variance (SAS 9.4, SAS Institute Inc., Cary, NC,  
142 USA). The mean values and standard errors of the means were presented as the results. Differences with a  
143 significance level of 0.05 were determined by the Student-Newman-Keuls multiple range test. Partial  
144 least squares-discriminant analysis was conducted using MetaboAnalyst 5.0 ([www.metaboanalyst.ca](http://www.metaboanalyst.ca)).

145

146

## 147 **Results and Discussion**

### 148 **Se content**

149

150 Throughout all storage days, the pork loin supplemented with Se45 showed the highest Se contents  
151 followed by Se15 and Con (Fig. 1;  $p=0.0009$ ). This indicates that the higher organic Se supplementation  
152 leads to higher residual Se contents in pork loins, as organic Se sources are highly bioavailable [15, 29].  
153 When Se-yeast was supplied as organic Se source, the amount of Se in the loin increased with increasing  
154 Se concentration in the feed [30]. Zhan et al. [22] also confirmed that pig muscle Se content increased  
155 more than double when fed with organic Se compared to inorganic Se. According to the findings of  
156 Zhang et al. [31], intramuscular Se content increased significantly when SeMet was used as a feed source,  
157 in comparison to inorganic Se sources such as SeNa or basic feeding treatment groups. Furthermore,  
158 organic Se has low toxicity, high transfer efficiency, and the ability to build and maintain Se reserve in  
159 muscle [30].

160 Meanwhile, Se contents were slightly decreased in Se15 and Se45 on day 7 and remained constant  
161 thereafter (Fig. 1;  $p<0.0001$ ). This reduction in Se content in pork during the refrigerated storage is likely  
162 due to microbial activity, temperature, etc. [32]. Despite this decrease, Se15 and Se45 still had higher Se  
163 contents than Con, indicating that the effect of Se supplementation can be maintained in pork during  
164 storage. We found no further impact from the interaction between the treatment and storage period  
165 ( $p=0.6826$ ).

166 The increased Se content in pork can have various impacts, as Se may have prevented oxidative  
167 damage from live animals to meat storage [33]. Therefore, high productivity can be promoted for pigs,

168 consumers who lack selenium can be relieved, and several beneficial effects can be provided to  
169 consumers. Se supplementation in live animals can improve reproductive physiological characteristics,  
170 such as semen volume and semen concentration [34]. Furthermore, Se supplementation in live animals  
171 can improve reproductive physiological characteristics, such as semen volume and semen concentration  
172 [34]. Furthermore, Se content in milk from sow increases, which has the advantage of solving Se  
173 deficiency that can easily occur in piglets [19]. With regards to meat quality, the supplementation of  
174 organic Se can enhance meat color stability by protecting myoglobin from oxidation with its antioxidant  
175 ability [22]. Calvo et al. [35] confirmed that Se-fed pork has high lipid stability during storage. In  
176 addition, consumption of Se-enriched pork may result in a reduction in toxic factors, as Se in pork has the  
177 ability to bind with heavy metals (such as cadmium, mercury, zinc, etc.) and facilitate their excretion from  
178 the body [36, 37]. Moreover, Se content in pork exhibits antioxidant effects by interacting with various  
179 antioxidant enzymes in the body, which can prevent DNA damage by averting several harmful effects of  
180 free radicals [38]. Therefore, when higher organic Se is fed to pigs, pork with the higher Se content can be  
181 served to consumers, providing additional health benefits at the point of their consumption.

182

### 183 **Antioxidant properties**

184

#### 185 **GPx activity**

186 GPx is an antioxidant enzyme that contains Se [9, 39] and can be increased by Se supplementation in  
187 pigs [40, 41]. As a result of confirming GPx activity in this study, organic Se supplementation had a  
188 significant effect on GPx activity (Fig. 2;  $p=0.0179$ ), but the effect of interaction between organic Se  
189 supplementation and storage period was not confirmed ( $p=0.7874$ ). Previous research has indicated that  
190 selenium can be absorbed through the digestive system and subsequently accumulated in various organs  
191 [6]. The accumulated Se undergoes various metabolic processes and plays a key role in the synthesis of  
192 GPx. As GPx contains Se in its active center, increased uptake and accumulation of Se in the body can  
193 promote its activity [42].

194 The increased activity of antioxidant enzymes may improve the storage stability of meat. Although the  
195 Se content in muscle decreased as the storage days increased in the experimental groups fed Se, Se45 had  
196 the highest Se content on all storage days. The increased activity of antioxidant enzymes can increase the  
197 antioxidant capacity of meat, which can have a positive effect on improving meat quality such as storage  
198 stability.

199

#### 200 **ABTS and DPPH scavenging activities**

201 To investigate antioxidant capacity of Se-supplemented pork, ABTS/DPPH scavenging activities were  
202 conducted (Table 1). Organic Se supplementation did not significantly change the ABTS scavenging  
203 activity, the DPPH scavenging activity showed a similar trend in each treatment, possibly due to their



204 strong correlation ( $r=0.906$ ). These unexpected results could be attributed to the fact that the change in  
205 GPx activity was not sufficient to affect the antioxidant activity of meat (Figs. 1 and 2). Although GPx  
206 plays a role in reducing lipid peroxide to alcohol and free hydrogen peroxide to water [43], ABTS/DPPH  
207 scavenging activities confirm the antioxidant effect through scavenging of free radicals, not hydrogen  
208 peroxide, and may not directly related to the high activity of GPx.

209 During 14 days of storage period, the tendencies in DPPH and ABTS scavenging activities were  
210 different (Table 1). ABTS scavenging activity was gradually increased, possibly due to the increased  
211 functional peptides from protein degradation during post-mortem ( $p<0.05$ ) [44]. However, in the case of  
212 DPPH assay, its activity was significantly decreased on day 7 and increased thereafter. The different  
213 results in ABTS and DPPH scavenging activities may be attributed to different mechanisms and subjects  
214 of both analytical methods. The ABTS assay is for both hydrophilic and lipophilic antioxidants, whereas  
215 DPPH assay is more applicable to hydrophobic system. It seems that post-mortem changes in pork  
216 induced stronger impact on ABTS and DPPH scavenging activities than that from organic Se  
217 supplementation.

218

### 219 **TBARS**

220 Lipid oxidation is a major concern in pork quality, as it can negatively affect acceptability of the meat.  
221 The oxidation of lipids can occur due to the inadequate scavenging capacity of antioxidants against the  
222 release of free radicals [45]. The extent of lipid oxidation during storage was assessed by conducting  
223 TBARS analysis as shown in Table 1. In the present study, organic Se supplementation did not exhibit a  
224 significant impact on lipid oxidation compared to the control group. This was unexpected as meat GPx  
225 activity can counteract free radicals, thereby influencing lipid oxidation [46]. Several factors may have  
226 contributed to this finding. Firstly, slow lipid oxidation rate by low-fat content in pork loin may have  
227 made it difficult to observe the differences from the enhanced GPx activity in the Se-supplemented  
228 groups (Fig. 2), as fat content is one of the main factors affecting lipid oxidation [45]. Additionally, the  
229 progress of lipid oxidation may have been delayed as the samples were stored at low temperatures.  
230 Consequently, we found that the lipid oxidation barely occurred in all groups after 14 days of storage,  
231 regardless of different Se feedings (Table 1). On day 7, a slight but significant decrease in TBARS value  
232 was found only in the Se-supplemented groups. Secondly, the increase in GPx may not have been enough  
233 to inhibit further lipid oxidation in pork loin. Hoac et al. [47] reported a certain decrease in lipid oxidation  
234 by GPx activity when 4 U/g GPx was added to chickens and ducks.

235 Taken the results from antioxidant properties together, although Se supplementation improved the  
236 activity of GPx, these changes did not affect the antioxidant activity and the lipid stability of pork loin  
237 during storage.

238

### 239 **Physicochemical properties**

240

## 241 **WHC and pH**

242 During the storage period, no significant difference was observed in WHC and pH between the control  
243 and groups supplemented with organic Se (Table 2;  $p=0.5897$  and  $p=0.2557$ , respectively). However, the  
244 changes in these properties varied depending on the levels of organic Se supplementation. During 14 days  
245 of storage, the WHC changed by 13.59, 18.79, and 18.89% in the control, Se15, and Se45 groups,  
246 respectively. It can be attributed to the decrease in water content over time (data not shown), as its  
247 decrease may limit free water release [48]. Similarly, the pH decreased at different rates in each group,  
248 with the control group having a decrease of 0.39, while Se15 and Se45 had reduction of 0.26 and 0.24,  
249 respectively. Even though several studies have reported that organic Se supplementation can increase  
250 WHC and reduce the decrease in pH in pork after slaughter [33, 49], in this study, organic Se  
251 supplementation (15 or 45 ppm) with 10 ppm inorganic Se did not affect WHC and pH in pork during 14  
252 days of storage.

253

## 254 **Meat color**

255 In regards to meat color, there was no significant difference in the CIE L<sup>\*</sup>-, a<sup>\*</sup>-, and b<sup>\*</sup>-values among  
256 different organic Se supplementation, except for a<sup>\*</sup>-value on day 7 (Table 3). While previous studies have  
257 reported that organic Se supplementation at 0.3 ppm can increase a<sup>\*</sup> and b<sup>\*</sup> values [35], this study did not  
258 observe any changes in meat color due to the lack of pH change in pork. The pH plays an important role  
259 in the mechanism by which oxymyoglobin is oxidized to metmyoglobin. In the case of Se-yeast, a type of  
260 organic Se fed in this experiment, it was absorbed through the methionine transporter and incorporated  
261 into the protein constituting the body, suggesting that it may not have affected meat quality, including its  
262 color. Nevertheless, previous research has indicated that consumption of organic Se may enhance muscle  
263 antioxidant capacity, protecting myoglobin from oxidation and thereby improving color stability [22].  
264 Conversely, inorganic Se has been reported to induce lighter color than pigs fed with organic Se, mainly  
265 due to water droplet loss that occurred when fed with inorganic Se [21].

266 During storage, different atmospheres can cause variation in the meat color of pork can [48]. The total  
267 color difference ( $\Delta E$ ) was calculated to confirm the changes in color (Table 3). Overall, no distinct color  
268 changes were observed in this study, indicating that the organic Se supplementation did not affect meat  
269 color in pork loin. The L<sup>\*</sup>-value tended to decrease, possibly due to an increase in WHC (Table 2),  
270 regardless of the type of organic Se supplementation. The a<sup>\*</sup>-value in each group was also affected by  
271 post-mortem changes. Its increases on day 7 is possibly due to the oxygenation of myoglobin and the  
272 value decreased due to oxidation to metmyoglobin [50].

273 No previous study has investigated the effect of mixed feeding of organic and inorganic Se on  
274 the meat color of pork. Based on the results of this study, the organic Se supplementation  
275 treatment did not affect meat color.

276

### 277 **NMR-based metabolic analysis**

278

279 We performed NMR-based metabolic analysis to investigate the effects of different Se supplementation  
280 on the metabolic profiles of pork loin during 14 days of storage. Table 4 presents a total of 31 metabolites  
281 that were identified across all groups, including 15 free amino acids, 4 nucleotide-related products, and 3  
282 organic acids. To assess the metabolomic differences among treatment groups and storage periods,  
283 multivariate analysis was performed, as shown in Figures 3 and 4, respectively. The metabolic profiles of  
284 Con, Se15, and Se45 were not distinctly different from each other on each storage day, as indicated in  
285 Figure 3. This suggests that the accumulated Se content in Se15 and Se45 did not have an impact on the  
286 metabolic differences during the storage period. No significant changes in metabolites, except for a few  
287 such as tyrosine, inosine, and betaine on day 0 and glutamate on day 14, were observed with different Se  
288 supplementation. Furthermore, lactate content was not significantly different between Con and both Se-  
289 supplemented groups (Table 4), but its content increased during storage, leading to a pH decrease (Table  
290 2). Although slight changes in the metabolites in each group were observed during storage period, in  
291 overall, these changes were not distinct (Fig. 4). Each group exhibited different changes in the levels of  
292 amino acids (alanine, asparagine, creatine, glutamate, glutamine, glycine, isoleucine, leucine, methionine,  
293 phenylalanine, threonine, tyrosine, and valine) and nucleotide-related compounds (hypoxanthine and  
294 inosine), as shown in Table 4. These changes can be attributed to the degradation of proteins and nucleic  
295 acids during storage, leading to an increase in the content of degradation products [51]. Additionally,  
296 lactate, which was previously mentioned, the other metabolites (acetate, carnosine, ethanol, glucose, N,N-  
297 dimethylglycine, niacinamide, and O-acetylcarnitine) also showed significant changes during 14 days of  
298 storage, but not due to Se supplementation. These results suggest that the Se feeding conditions used in  
299 this experiment were not sufficient to induce metabolomic changes in pork loin.

300

301

302

## 302 **Conclusion**

303 This study found that different levels of organic Se (0.15 and 0.45 ppm) combined with inorganic Se  
304 did not significantly affect pork quality during 14 days of storage, despite an increase in tissue Se content  
305 and GPx activity. Therefore, high Se content in the organic Se-fed group may have a positive effect on Se  
306 accumulation in pig muscle, but organic Se supplementation up to 45 ppm does not affect pork quality  
307 during storage periods of up to 14 days. In the results of supplementation with Se, the same phenomenon

308 as the control group was confirmed on all days of storage. Therefore, through this study, it was confirmed  
309 that Se, a trace mineral used for pig breeding management, does not adversely affect pork quality.

310

311

312

## 313 **References**

- 314 1. Schwarz T, Przybyło M, Zapletal P, Turek A, Pabiańczyk M, Bartlewski PM. Effects of using corn  
315 dried distillers' grains with solubles (cDDGS) as a partial replacement for soybean meal on the  
316 outcomes of pig fattening, pork slaughter value and quality. *Animals*. 2021;11:10.  
317 <https://doi.org/10.3390/ani11102956>
- 318 2. Lebret B. Effects of feeding and rearing systems on growth, carcass composition and meat quality in  
319 pigs. *Animal*. 2008;2:1548-1558. <https://doi.org/10.1017/S1751731108002796>
- 320 3. Kouba M, Sellier P. A review of the factors influencing the development of intermuscular adipose  
321 tissue in the growing pig. *Meat Science*. 2011;88:213-220.  
322 <https://doi.org/10.1016/j.meatsci.2011.01.003>
- 323 4. Jiang J, Xiong YL. Natural antioxidants as food and feed additives to promote health benefits and  
324 quality of meat products: A review. *Meat Science*. 2016;120:107-117.  
325 <https://doi.org/10.1016/j.meatsci.2016.04.005>
- 326 5. Rosenvold K, Andersen HJ. Factors of significance for pork quality—a review. *Meat Science*.  
327 2003;64:219-237. [https://doi.org/10.1016/S0309-1740\(02\)00186-9](https://doi.org/10.1016/S0309-1740(02)00186-9)
- 328 6. Dalgaard TS, Briens M, Engberg RM, Lauridsen C. The influence of selenium and selenoproteins on  
329 immune responses of poultry and pigs. *Animal Feed Science and Technology*. 2018;238:73-83.  
330 <https://doi.org/10.1016/j.anifeedsci.2018.01.020>
- 331 7. Surai PF, Fisinin VI. Selenium in Pig Nutrition and reproduction: Boars and semen quality—A Review.  
332 *Asian-Australasian Journal of Animal Sciences*. 2015;28:730. <https://doi.org/10.5713/ajas.14.0593>
- 333 8. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: biochemical  
334 role as a component of glutathione peroxidase. *Science*. 1973;179:588-590.  
335 <https://doi.org/10.1126/science.179.4073.588>
- 336 9. Surai PF. Selenium in nutrition and health. 1st ed. Nottingham: Nottingham university press; 2006
- 337 10. Muthukumar K, Rajakumar S, Sarkar MN, Nachiappan V. Glutathione peroxidase3 of *Saccharomyces*  
338 *cerevisiae* protects phospholipids during cadmium-induced oxidative stress. *Antonie Van*  
339 *Leeuwenhoek*. 2011;99:761-771. <https://doi.org/10.1007/s10482-011-9550-9>
- 340 11. Chen J, Tian M, Guan W, Wen T, Yang F, Chen F, Zhang S, Song J, Ren C, Zhang Y, Song H.  
341 Increasing selenium supplementation to a moderately-reduced energy and protein diet improves  
342 antioxidant status and meat quality without affecting growth performance in finishing pigs. *Journal*  
343 *of Trace Elements in Medicine and Biology*. 2019;56:38-45.  
344 <https://doi.org/10.1016/j.jtemb.2019.07.004>

- 345 12. Tinggi U. Selenium: its role as antioxidant in human health. *Environmental Health and Preventive*  
346 *Medicine*. 2008;13:102-108. <https://doi.org/10.1007/s12199-007-0019-4>
- 347 13. Wei C, Lin X, Zhang Y, Wan X, Wu H, He T, Bi K, Wang C. Effects of inorganic and organic  
348 selenium sources on the growth performance of broilers in China: A meta-analysis. *Open Life*  
349 *Sciences*. 2021;16:31-38. <https://doi.org/10.1515/biol-2021-0007>
- 350 14. Kim YY. Differences in biological activity and metabolism of selenium due to its chemical form. *J*  
351 *Anim Sci Technol*. 2000;42:835-848.
- 352 15. Mahan DC, Parrett NA. Evaluating the efficacy of selenium-enriched yeast and sodium selenite on  
353 tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine.  
354 *Journal of Animal Science*. 1996;74:2967-2974. <https://doi.org/10.2527/1996.74122967x>
- 355 16. Kim YY, Mahan DC. Comparative effects of high dietary levels of organic and inorganic selenium on  
356 selenium toxicity of growing-finishing pigs. *Journal of Animal Science*. 2001;79:942-948.  
357 <https://doi.org/10.2527/2001.794942x>
- 358 17. Lawler TL, Taylor JB, Finley JW, Caton JS. Effect of supranutritional and organically bound  
359 selenium on performance, carcass characteristics, and selenium distribution in finishing beef steers.  
360 *Journal of Animal Science*. 2004;82:1488-1493. <https://doi.org/10.2527/2004.8251488x>
- 361 18. Mahan DC, Azain M, Crenshaw TD, Cromwell GL, Dove CR, Kim SW, Lindemann MD, Miller PS,  
362 Pettigrew JE, Stein HH, Van Heugten E. Supplementation of organic and inorganic selenium to diets  
363 using grains grown in various regions of the United States with differing natural Se concentrations  
364 and fed to grower–finisher swine. *Journal of Animal Science*. 2014;92:4991-4997.  
365 <https://doi.org/10.2527/jas.2014-7735>
- 366 19. Mahan DC, Kim YY. Effect of inorganic or organic selenium at two dietary levels on reproductive  
367 performance and tissue selenium concentrations in first-parity gilts and their progeny. *Journal of*  
368 *Animal Science*. 1996;74:2711-2718. <https://doi.org/10.2527/1996.74112711x>
- 369 20. Morrissey PA, Sheehy PJA, Galvin K, Kerry JP, Buckley DJ. Lipid stability in meat and meat  
370 products. *Meat Science*. 1998;49:73-86. [https://doi.org/10.1016/S0309-1740\(98\)90039-0](https://doi.org/10.1016/S0309-1740(98)90039-0)
- 371 21. Mahan DC, Cline TR, Richert B. Effects of dietary levels of selenium-enriched yeast and sodium  
372 selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum  
373 glutathione peroxidase activity, carcass characteristics, and loin quality. *Journal of Animal Science*.  
374 1999;77:2172-2179. <https://doi.org/10.2527/1999.7782172x>
- 375 22. Zhan X, Wang M, Zhao R, Li W, Xu Z. Effects of different selenium source on selenium distribution,  
376 loin quality and antioxidant status in finishing pigs. *Animal Feed Science and Technology*.  
377 2007;132:202-211. <https://doi.org/10.1016/j.anifeedsci.2006.03.020>

- 378 23. Gjerlaug-Enger E, Haug A, Gaarder M, Ljøkjel K, Stenseth RS, Sigfridson K, Egelanddal B, Saarem  
379 K, Berg P. Pig feeds rich in rapeseed products and organic selenium increased omega-3 fatty acids  
380 and selenium in pork meat and backfat. *Food Science and Nutrition*. 2015;3:120-128.  
381 <https://doi.org/10.1002/fsn3.182>
- 382 24. Khalili M, Chamani M, Amanlou H, Nikkhah A, Sadeghi AA, Dehkordi FK, Rafiei M, Shirani V. The  
383 effect of feeding inorganic and organic selenium sources on the hematological blood parameters,  
384 reproduction and health of dairy cows in the transition period. *Acta Scientiarum. Animal Sciences*.  
385 2019;42. <https://doi.org/10.4025/actascianimsci.v42i1.45371>
- 386 25. Wang Z, Kong L, Zhu L, Hu X, Su P, Song Z. The mixed application of organic and inorganic  
387 selenium shows better effects on incubation and progeny parameters. *Poultry Science*.  
388 2021;100:1132-1141. <https://doi.org/10.1016/j.psj.2020.10.037>
- 389 26. Choe J, Park B, Lee HJ, Jo C. Potential antioxidant and angiotensin I-converting enzyme inhibitory  
390 activity in crust of dry-aged beef. *Scientific Reports*, 2020;10;7883. <https://doi.org/10.1038/s41598-020-64861-0>
- 392 27. Rupasinghe R, Alahakoon AU, Alakolanga AW, Jayasena DD, Jo C. Oxidative stability of vacuum-  
393 packed chicken wings marinated with fruit juices during frozen storage. *Food Science of Animal*  
394 *Resources*, 2021;42;61-72. <https://doi.org/10.5851/kosfa.2021.e62>
- 395 28. Kwon JA, Yim DG, Kim HJ, Ismail A, Kim SS, Lee HJ, Jo C. Effect of temperature abuse on quality  
396 and metabolites of frozen/thawed beef loins. 2022;42;341-349. <https://doi.org/10.5851/kosfa.2022.e9>
- 397 29. Kim HC, Yim DG, Kim JW, Lee D, Jo C. Nuclear magnetic resonance (NMR)-based quantification  
398 on flavor-active and bioactive compounds and application for distinguishment of chicken breeds.  
399 *Food Science of Animal Resources*. 2021;41: 312. <https://doi.org/10.5851/kosfa.2020.e102>
- 400 30. Burk RF, Hill KE. Regulation of selenium metabolism and transport. *Annual review of nutrition*.  
401 2015;35:109-134. <https://doi.org/10.1146/annurev-nutr-071714-034250>
- 402 31. Zhang K, Zhao Q, Zhan T, Han Y, Tang C, Zhang J. Effect of different selenium sources on growth  
403 performance, tissue selenium content, meat quality, and selenoprotein gene expression in finishing  
404 pigs. *Biological Trace Element Research*. 2020;196:463-471. <https://doi.org/10.1007/s12011-019-01949-3>
- 406 32. Sentkowska A, Pyrzynska K. Stability of selenium compounds in aqueous extracts of dietary  
407 supplements during storage. *Journal of Pharmaceutical and Biomedical Analysis*. 2022;214:114714.  
408 <https://doi.org/10.1016/j.jpba.2022.114714>
- 409 33. Calvo L, Toldrá F, Aristoy MC, López-Bote CJ, Rey AI. Effect of dietary organic selenium on muscle  
410 proteolytic activity and water-holding capacity in pork. *Meat Science*. 2016;121:1-11.  
411 <https://doi.org/10.1016/j.meatsci.2016.05.006>

- 412 34. Martin-Guzman J, Mahan DC, Chung YK, Pate JL, Pope WF. Effects of dietary selenium and vitamin  
413 E on boar performance and tissue response semen quality and subsequent fertilisation rate in mature  
414 gilts. *J Anim Sci.* 1997;75:2994-3003. <https://doi.org/10.2527/1997.75112994x>
- 415 35. Calvo L, Toldrá F, Rodríguez AI, López-Bote C, Rey AI. Effect of dietary selenium source (organic  
416 vs. mineral) and muscle pH on meat quality characteristics of pigs. *Food Science and Nutrition.*  
417 2017;5:94-102. <https://doi.org/10.1002/fsn3.368>
- 418 36. Feroci G, Badiello R, Fini A. Interactions between different selenium compounds and zinc, cadmium  
419 and mercury. *Journal of Trace Elements in Medicine and Biology.* 2015;18:227-234.  
420 <https://doi.org/10.1016/j.jtemb.2004.09.005>
- 421 37. Kieliszek M, Błażej S. Current knowledge on the importance of selenium in food for living  
422 organisms: a review. *Molecules.* 2016;21:609. <https://doi.org/10.3390/molecules21050609>
- 423 38. Karag E, Németh I, Ferke A, Hajdú J, Pintér S. A vörösvértest szelén és antagonistá nyomelemek,  
424 valamint a plazma antioxidánsok koncentrációja és összefüggése érett újszülöttek köldökzsínór  
425 vérében. Cser MÁ, Sziklai-László I.,(szerk.): *A Szelén Szerepe a Környezetben és*  
426 *Egészségvédelemben.* 1998;112-114.
- 427 39. Rotruck JT, Pope AL, Ganther HE. Selenium biochemical role as a component of GPx purification  
428 assay. *Science.* 1973;179:588-590.
- 429 40. Jiang J, Tang X, Xue Y, Lin G, Xiong YL. Dietary linseed oil supplemented with organic selenium  
430 improved the fatty acid nutritional profile, muscular selenium deposition, water retention, and  
431 tenderness of fresh pork. *Meat Science.* 2017;131: 99-106.  
432 <https://doi.org/10.1016/j.meatsci.2017.03.014>
- 433 41. Marković R, Ćirić J, Drljačić A, Šefer D, Jovanović I, Jovanović D, Milanović S, Trbović D,  
434 Radulović S, Baltić MŽ, Starčević M. The effects of dietary Selenium-yeast level on glutathione  
435 peroxidase activity, tissue Selenium content, growth performance, and carcass and meat quality of  
436 broilers. *Poultry Science.* 2018;97: 2861-2870. <https://doi.org/10.3382/ps/pey117>
- 437 42. Navarro-Alarcon M, López-Martinez MC. Essentiality of selenium in the human body: relationship  
438 with different diseases. *Science of the Total Environment.* 2000;249:347-371.  
439 [https://doi.org/10.1016/S0048-9697\(99\)00526-4](https://doi.org/10.1016/S0048-9697(99)00526-4)
- 440 43. Muthukumar K, Rajakumar S, Sarkar MN, Nachiappan V. Glutathione peroxidase3 of *Saccharomyces*  
441 *cerevisiae* protects phospholipids during cadmium-induced oxidative stress. *Antonie Van*  
442 *Leeuwenhoek.* 2011;99:761-771. <https://doi.org/10.1007/s10482-011-9550-9>
- 443 44. Sohaib M, Anjum FM, Sahar A, Arshad MS, Rahman UU, Imran A, Hussain S. Antioxidant proteins  
444 and peptides to enhance the oxidative stability of meat and meat products: A comprehensive review.



445 International Journal of Food Properties. 2017;20:2581-2593.  
446 <https://doi.org/10.1080/10942912.2016.1246456>

447 45. Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on  
448 lipid oxidation in meat and meat products. *Antioxidants*. 2019;8:429.  
449 <https://doi.org/10.3390/antiox8100429>

450 46. Surai PF, Kochish II, Fisinin VI, Juniper DT. Revisiting oxidative stress and the use of organic  
451 selenium in dairy cow nutrition. *Animals*. 2019;9:462. <https://doi.org/10.3390/ani9070462>

452 47. Hoac T, Daun C, Trafikowska U, Zackrisson J, Åkesson B. Influence of heat treatment on lipid  
453 oxidation and glutathione peroxidase activity in chicken and duck meat. *Innovative Food Science  
454 and Emerging Technologies*. 2006;7:88-93. <https://doi.org/10.1016/j.ifset.2005.10.001>

455 48. Szymańko T, Lesiów T, Górecka J. The water-holding capacity of meat: A reference analytical method.  
456 *Food Chemistry*. 2021;357:129727. <https://doi.org/10.1016/j.foodchem.2021.129727>

457 49. Li JG, Zhou JC, Zhao H, Lei XG, Xia XJ, Gao G, Wang KN. Enhanced water-holding capacity of  
458 meat was associated with increased *Sepw1* gene expression in pigs fed selenium-enriched yeast.  
459 *Meat Science*. 2011;87:95-100. <https://doi.org/10.1016/j.meatsci.2010.05.019>

460 50. Viana ES, Gomide LAM, Vanetti MCD. Effect of modified atmospheres on microbiological, color  
461 and sensory properties of refrigerated pork. *Meat Science*. 2005;71:696-705.  
462 <https://doi.org/10.1016/j.meatsci.2005.05.013>

463 51. Tamura Y, Iwatoh S, Miyaura K, Asikin Y, Kusano M. Metabolomic profiling reveals the relationship  
464 between taste-related metabolites and roasted aroma in aged pork. *LWT*. 2022;155:112928.  
465 <https://doi.org/10.1016/j.lwt.2021.112928>

466

467

468

469

470 **Table 1.** Antioxidant properties of pork loin as raised under different selenium supplementation  
 471 conditions and storage period.

Item	Treatment	Storage period (days)			SEM <sup>1</sup>
		0	7	14	
TBARS (mg MDA/kg)	Con	0.18	0.15	0.18	0.016
	Se15	0.18 <sup>a</sup>	0.13 <sup>b</sup>	0.18 <sup>a</sup>	0.011
	Se45	0.16 <sup>ab</sup>	0.12 <sup>b</sup>	0.18 <sup>a</sup>	0.015
	SEM <sup>2</sup>	0.021	0.009	0.010	
ABTS scavenging rate (%)	Con	32.59 <sup>b</sup>	39.79 <sup>a</sup>	39.63 <sup>a</sup>	1.815
	Se15	31.28 <sup>c</sup>	36.70 <sup>b</sup>	42.31 <sup>a</sup>	1.233
	Se45	33.11 <sup>b</sup>	42.46 <sup>a</sup>	44.79 <sup>a</sup>	1.495
	SEM <sup>2</sup>	0.948	1.987	1.484	
DPPH scavenging rate (%)	Con	82.42 <sup>a</sup>	60.56 <sup>c</sup>	68.26 <sup>b</sup>	2.267
	Se15	81.06 <sup>a</sup>	59.89 <sup>c</sup>	68.46 <sup>b</sup>	1.473
	Se45	83.72 <sup>a</sup>	61.87 <sup>c</sup>	71.36 <sup>b</sup>	1.180
	SEM <sup>2</sup>	1.530	1.901	1.657	

472 Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic  
 473 Se 0.45 ppm + inorganic Se 0.10 ppm; TBARS, 2-thiobarbituric acid reactive substances; ABTS, 2,2'-azinobis-(3-  
 474 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl

475 <sup>1</sup> Standard error of mean (n = 15).

476 <sup>2</sup> Standard error of mean (n = 15).

477 <sup>A-C</sup> Different letters within the same column indicate significant differences (P < 0.05).

478 <sup>a-c</sup> Different letters within the same row differ significantly (P < 0.05).

479

480 **Table 2.** Water holding capacity (WHC) and pH of pork loin raised under different selenium  
 481 supplementation conditions and storage period.

Item	Treatment	Storage period (days)			SEM <sup>1</sup>
		0	7	14	
WHC (%)	Con	59.35 <sup>b</sup>	61.50 <sup>b</sup>	72.94 <sup>a</sup>	2.414
	Se15	57.80 <sup>b</sup>	65.27 <sup>b</sup>	76.59 <sup>a</sup>	2.681
	Se45	55.37 <sup>c</sup>	61.06 <sup>b</sup>	74.26 <sup>a</sup>	1.289
	SEM <sup>2</sup>	2.319	2.336	1.960	
pH	Con	5.90 <sup>a</sup>	5.53 <sup>b</sup>	5.51 <sup>b</sup>	0.058
	Se15	5.79 <sup>a</sup>	5.50 <sup>b</sup>	5.53 <sup>b</sup>	0.050
	Se45	5.81 <sup>a</sup>	5.54 <sup>b</sup>	5.57 <sup>b</sup>	0.048
	SEM <sup>2</sup>	0.067	0.047	0.038	

482 Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic  
 483 Se 0.45 ppm + inorganic Se 0.10 ppm; WHC, water holding capacity

484 <sup>1</sup> Standard error of mean (n = 15).

485 <sup>2</sup> Standard error of mean (n = 15).

486 <sup>A-C</sup> Different letters within the same column indicate significant differences (P < 0.05).

487 <sup>a-c</sup> Different letters within the same row differ significantly (P < 0.05).

488

489 **Table 3.** Meat color of pork loin raised under different selenium supplementation conditions and  
 490 storage period.

Item	Treatment	Storage period (days)			SEM <sup>1</sup>
		0	7	14	
CIE L*	Con	55.56	54.47	50.93	1.353
	Se15	54.77 <sup>a</sup>	55.14 <sup>a</sup>	48.85 <sup>b</sup>	0.833
	Se45	54.63 <sup>ab</sup>	57.94 <sup>a</sup>	51.17 <sup>b</sup>	1.120
	SEM <sup>2</sup>	0.816	1.320	1.247	
CIE a*	Con	6.70 <sup>b</sup>	11.26 <sup>ABa</sup>	10.41 <sup>a</sup>	0.636
	Se15	6.78 <sup>c</sup>	12.04 <sup>Aa</sup>	10.28 <sup>b</sup>	0.565
	Se45	6.76 <sup>b</sup>	10.03 <sup>Ba</sup>	9.05 <sup>a</sup>	0.585
	SEM <sup>2</sup>	0.431	0.529	0.775	
CIE b*	Con	13.10 <sup>c</sup>	17.11 <sup>a</sup>	15.49 <sup>b</sup>	0.509
	Se15	13.05 <sup>c</sup>	17.87 <sup>a</sup>	14.89 <sup>b</sup>	0.317
	Se45	12.09 <sup>c</sup>	16.31 <sup>a</sup>	14.15 <sup>b</sup>	0.593
	SEM <sup>2</sup>	0.285	0.676	0.415	
Chroma	Con	14.74 <sup>b</sup>	20.52 <sup>a</sup>	18.73 <sup>a</sup>	0.631
	Se15	14.75 <sup>c</sup>	21.60 <sup>a</sup>	18.14 <sup>b</sup>	0.525
	Se45	13.86 <sup>c</sup>	19.19 <sup>a</sup>	16.85 <sup>b</sup>	0.747
	SEM <sup>2</sup>	0.371	0.792	0.683	
Hue angle	Con	62.97 <sup>a</sup>	56.73 <sup>b</sup>	56.48 <sup>b</sup>	1.649
	Se15	62.56 <sup>a</sup>	56.32 <sup>b</sup>	55.66 <sup>b</sup>	1.269
	Se45	60.87	58.57	57.67	1.290
	SEM <sup>2</sup>	1.386	0.965	1.773	
ΔE	Con	-	7.50	6.69	1.437
	Se15	-	7.21	7.30	0.985
	Se45	-	6.57	5.47	1.221
	SEM <sup>2</sup>	-	1.075	1.362	

491 Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic  
 492 Se 0.45 ppm + inorganic Se 0.10 ppm

493 <sup>1</sup> Standard error of mean (n = 15).

494 <sup>2</sup> Standard error of mean (n = 15).

495 <sup>A-C</sup> Different letters within the same column indicate significant differences (P < 0.05).

496 <sup>a-c</sup> Different letters within the same row differ significantly (P < 0.05).

497

498 **Table 4.** Metabolites profiles (mg/100g) of pork loin raised under different selenium  
 499 supplementation conditions and storage period.  
 500

Item	Treatment	Storage period (days)			SEM <sup>1</sup>
		0	7	14	
<i>Free amino acids</i>					
Alanine	Con	29.67	22.10	28.83	2.752
	Se15	24.89 <sup>ab</sup>	22.15 <sup>b</sup>	31.60 <sup>a</sup>	2.291
	Se45	28.67	26.81	33.32	2.033
	SEM <sup>2</sup>	3.125	1.922	1.870	
Asparagine	Con	3.54 <sup>b</sup>	3.77 <sup>b</sup>	6.67 <sup>a</sup>	0.770
	Se15	2.93 <sup>b</sup>	4.11 <sup>ab</sup>	4.95 <sup>a</sup>	0.451
	Se45	3.21 <sup>b</sup>	4.64 <sup>ab</sup>	6.08 <sup>a</sup>	0.606
	SEM <sup>2</sup>	0.506	0.497	0.813	
Creatine	Con	391.88 <sup>b</sup>	431.87 <sup>b</sup>	509.16 <sup>a</sup>	16.008
	Se15	406.25 <sup>c</sup>	453.50 <sup>b</sup>	488.20 <sup>a</sup>	9.882
	Se45	410.58	476.93	504.61	30.326
	SEM <sup>2</sup>	10.728	30.223	15.654	
Glutamate	Con	7.08 <sup>b</sup>	8.34 <sup>b</sup>	12.49 <sup>Ba</sup>	1.179
	Se15	8.87 <sup>b</sup>	10.99 <sup>ab</sup>	14.211 <sup>ABa</sup>	1.284
	Se45	9.05 <sup>b</sup>	11.28 <sup>b</sup>	16.74 <sup>Aa</sup>	0.996
	SEM <sup>2</sup>	1.161	1.194	1.121	
Glutamine	Con	27.97 <sup>a</sup>	16.53 <sup>b</sup>	18.89 <sup>b</sup>	2.882
	Se15	25.37	17.02	18.51	3.409
	Se45	26.81	22.90	19.65	2.703
	SEM <sup>2</sup>	4.629	2.143	1.105	
Glycine	Con	26.71	34.43	41.64	7.313
	Se15	28.90 <sup>b</sup>	36.20 <sup>ab</sup>	43.24 <sup>a</sup>	3.604
	Se45	27.88	36.75	36.57	4.295
	SEM <sup>2</sup>	4.004	5.113	6.537	
Isoleucine	Con	2.47 <sup>b</sup>	4.25 <sup>b</sup>	7.40 <sup>a</sup>	0.868
	Se15	2.96 <sup>c</sup>	5.80 <sup>b</sup>	8.93 <sup>a</sup>	0.430
	Se45	3.48 <sup>b</sup>	5.24 <sup>b</sup>	8.43 <sup>a</sup>	0.786
	SEM <sup>2</sup>	0.269	0.728	0.977	
Leucine	Con	2.80 <sup>b</sup>	5.28 <sup>b</sup>	9.22 <sup>a</sup>	1.197
	Se15	4.18 <sup>c</sup>	7.12 <sup>b</sup>	11.27 <sup>a</sup>	0.667
	Se45	4.12 <sup>b</sup>	6.65 <sup>b</sup>	10.95 <sup>a</sup>	0.975
	SEM <sup>2</sup>	0.568	0.983	1.241	
Methionine	Con	5.59 <sup>b</sup>	6.82 <sup>b</sup>	11.39 <sup>a</sup>	1.250
	Se15	5.12 <sup>c</sup>	8.79 <sup>b</sup>	11.96 <sup>a</sup>	0.636
	Se45	5.97 <sup>b</sup>	8.97 <sup>b</sup>	12.71 <sup>a</sup>	1.107
	SEM <sup>2</sup>	0.345	1.186	1.290	
Phenylalanine	Con	2.68 <sup>c</sup>	5.13 <sup>b</sup>	7.96 <sup>a</sup>	0.743
	Se15	3.35 <sup>c</sup>	6.34 <sup>b</sup>	9.54 <sup>a</sup>	0.304
	Se45	3.83 <sup>b</sup>	5.92 <sup>b</sup>	9.25 <sup>a</sup>	0.722
	SEM <sup>2</sup>	0.192	0.653	0.838	
Taurine	Con	38.23	35.77	40.20	4.510
	Se15	36.53	40.09	43.24	3.619

	Se45	46.18	42.41	38.74	2.928
	SEM <sup>2</sup>	3.399	4.239	3.534	
Threonine	Con	6.30 <sup>c</sup>	9.82 <sup>b</sup>	12.72 <sup>a</sup>	0.920
	Se15	7.28	13.99	13.14	1.950
	Se45	7.71 <sup>b</sup>	11.35 <sup>a</sup>	13.83 <sup>a</sup>	1.073
	SEM <sup>2</sup>	0.429	2.217	0.839	
Tyrosine	Con	3.69 <sup>Bb</sup>	8.54 <sup>b</sup>	14.82 <sup>a</sup>	1.632
	Se15	4.45 <sup>Bc</sup>	10.18 <sup>b</sup>	16.50 <sup>a</sup>	0.708
	Se45	5.60 <sup>A</sup>	10.19	16.78	1.408
	SEM <sup>2</sup>	0.307	1.288	1.842	
Valine	Con	4.16 <sup>b</sup>	6.10 <sup>b</sup>	9.68 <sup>a</sup>	1.124
	Se15	4.78 <sup>c</sup>	7.94 <sup>b</sup>	11.87 <sup>a</sup>	0.627
	Se45	5.70 <sup>b</sup>	7.58 <sup>b</sup>	11.60 <sup>a</sup>	0.992
	SEM <sup>2</sup>	0.439	0.941	1.250	
β-alanine	Con	7.49	7.33	8.40 <sup>AB</sup>	0.591
	Se15	7.72	7.96	7.99 <sup>B</sup>	0.381
	Se45	7.84	9.05	9.55 <sup>A</sup>	0.498
	SEM <sup>2</sup>	0.393	0.641	0.420	
<i>Nucleotide-related products</i>					
Hypoxanthine	Con	11.43	9.47	12.92	1.168
	Se15	11.74 <sup>ab</sup>	10.15 <sup>b</sup>	13.40 <sup>a</sup>	0.709
	Se45	12.24	11.69	13.47	1.051
	SEM <sup>2</sup>	1.373	0.745	0.731	
IMP	Con	79.80	92.02	76.74	5.137
	Se15	89.49	90.69	73.91	5.777
	Se45	90.51	100.20	82.46	7.372
	SEM <sup>2</sup>	7.590	7.072	2.549	
Inosine	Con	37.95 <sup>Bb</sup>	54.53 <sup>b</sup>	75.34 <sup>a</sup>	6.024
	Se15	37.73 <sup>Bc</sup>	57.22 <sup>b</sup>	77.24 <sup>a</sup>	2.165
	Se45	42.24 <sup>Ac</sup>	60.48 <sup>b</sup>	74.93 <sup>a</sup>	4.201
	SEM <sup>2</sup>	0.820	4.769	5.934	
UMP	Con	2.94	3.68	2.95	0.212
	Se15	3.52	3.65	3.54	0.173
	Se45	3.20	3.48	3.16	0.232
	SEM <sup>2</sup>	0.215	0.217	0.188	
<i>Organic acids</i>					
Acetate	Con	3.41 <sup>b</sup>	4.73 <sup>b</sup>	6.55 <sup>a</sup>	0.434
	Se15	3.37 <sup>c</sup>	5.33 <sup>b</sup>	7.16 <sup>a</sup>	0.269
	Se45	3.99	5.33	5.95	0.529
	SEM <sup>2</sup>	0.223	0.467	0.522	
Lactate	Con	266.39 <sup>b</sup>	345.02 <sup>a</sup>	389.90 <sup>a</sup>	18.649
	Se15	284.39 <sup>b</sup>	360.10 <sup>a</sup>	384.63 <sup>a</sup>	13.010
	Se45	277.13 <sup>b</sup>	362.30 <sup>a</sup>	371.84 <sup>a</sup>	24.988
	SEM <sup>2</sup>	16.370	24.980	15.794	
Methylmalonate	Con	5.59 <sup>b</sup>	7.15 <sup>b</sup>	8.96 <sup>a</sup>	0.529
	Se15	6.15 <sup>b</sup>	8.04 <sup>a</sup>	8.79 <sup>a</sup>	0.297
	Se45	5.68 <sup>b</sup>	7.82 <sup>a</sup>	8.60 <sup>a</sup>	0.590
	SEM <sup>2</sup>	0.302	0.560	0.557	
<i>Others</i>					
Betaine	Con	34.96 <sup>B</sup>	30.79	30.13	2.225
	Se15	34.86 <sup>B</sup>	35.28	28.78	4.596
	Se45	46.01 <sup>A</sup>	44.94	38.50	3.970

	SEM <sup>2</sup>	2.740	3.920	4.355	
Carnosine	Con	224.98 <sup>b</sup>	313.86 <sup>a</sup>	357.50 <sup>a</sup>	15.515
	Se15	284.85	323.75	347.96	27.245
	Se45	221.90 <sup>b</sup>	315.65 <sup>a</sup>	337.96 <sup>a</sup>	28.333
	SEM <sup>2</sup>	28.347	27.062	15.810	
Ethanol	Con	0.88	1.78	2.28	0.391
	Se15	1.04 <sup>b</sup>	2.47 <sup>a</sup>	2.40 <sup>a</sup>	0.141
	Se45	1.04 <sup>b</sup>	2.19 <sup>a</sup>	2.25 <sup>a</sup>	0.285
	SEM <sup>2</sup>	0.107	0.253	0.423	
Glucose	Con	42.92	72.18	81.68	19.945
	Se15	46.56 <sup>b</sup>	74.86 <sup>ab</sup>	87.63 <sup>a</sup>	9.465
	Se45	67.56	89.19	77.10	25.165
	SEM <sup>2</sup>	17.404	18.664	21.666	
Glycerol	Con	9.06	9.29	10.76	1.339
	Se15	9.65	9.24	12.96	1.112
	Se45	11.32	10.64	11.31	0.627
	SEM <sup>2</sup>	0.829	0.892	1.393	
Methanol	Con	0.74 <sup>a</sup>	0.30 <sup>b</sup>	0.33 <sup>b</sup>	0.105
	Se15	0.61	0.38	0.35	0.082
	Se45	0.75	0.49	0.41	0.100
	SEM <sup>2</sup>	0.136	0.091	0.033	
N,N-Dimethylglycine	Con	1.93 <sup>b</sup>	2.27 <sup>b</sup>	2.81 <sup>a</sup>	0.158
	Se15	1.90 <sup>c</sup>	2.45 <sup>b</sup>	2.71 <sup>a</sup>	0.055
	Se45	2.01 <sup>b</sup>	2.58 <sup>ab</sup>	2.83 <sup>a</sup>	0.190
	SEM <sup>2</sup>	0.058	0.189	0.158	
Niacinamide	Con	4.55 <sup>b</sup>	6.70 <sup>a</sup>	7.69 <sup>a</sup>	0.471
	Se15	5.05 <sup>c</sup>	6.99 <sup>b</sup>	7.86 <sup>a</sup>	0.244
	Se45	5.16 <sup>b</sup>	6.91 <sup>a</sup>	7.60 <sup>a</sup>	0.537
	SEM <sup>2</sup>	0.351	0.564	0.358	
O-Acetylcarnitine	Con	7.56 <sup>a</sup>	2.60 <sup>b</sup>	3.46 <sup>b</sup>	0.783
	Se15	7.51	3.64	3.96	1.300
	Se45	7.66 <sup>a</sup>	4.56 <sup>b</sup>	4.14 <sup>b</sup>	0.764
	SEM <sup>2</sup>	1.541	0.658	0.280	

501 Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic

502 Se 0.45 ppm + inorganic Se 0.10 ppm

503 <sup>1</sup> Standard error of mean (n = 15).

504 <sup>2</sup> Standard error of mean (n = 15).

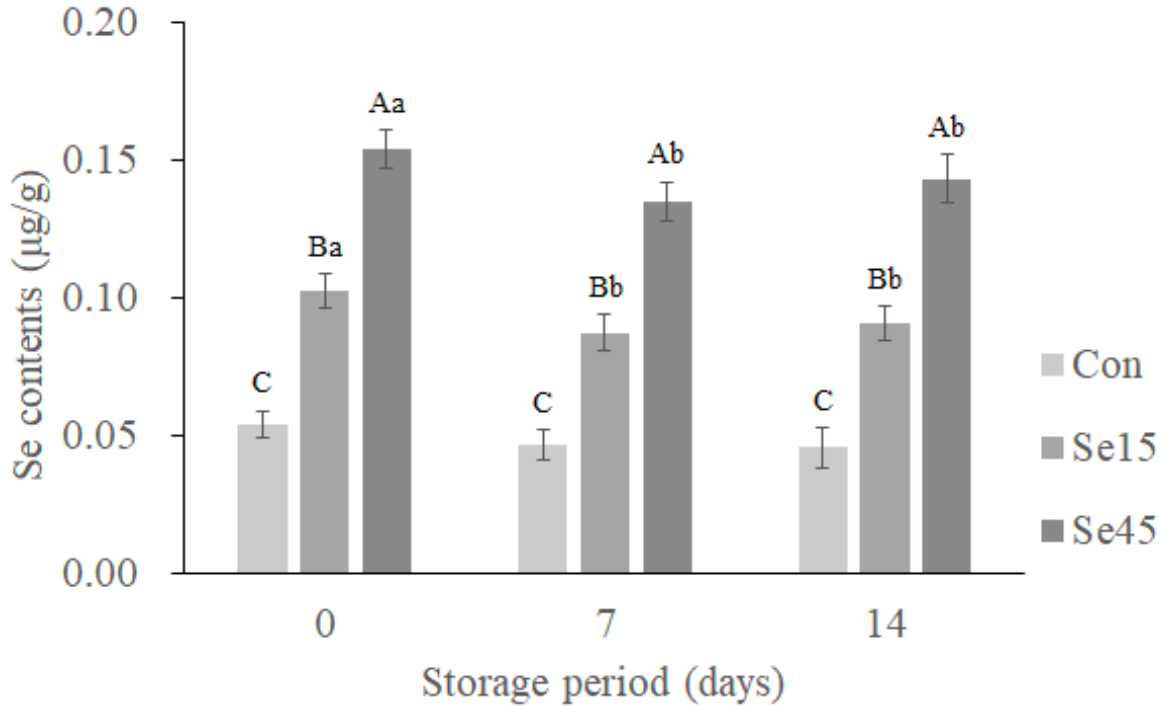
505 <sup>A-C</sup> Different letters within the same column indicate significant differences (P < 0.05).

506 <sup>a-c</sup> Different letters within the same row differ significantly (P < 0.05).

507

508 **Figure captions**

509



510

511 **Fig. 1.** Selenium contents of pork loin raised under different selenium supplementation conditions and  
512 storage period. Abbreviations: Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10  
513 ppm; Se45, pork loin from feeding organic Se 0.45 ppm + inorganic Se 0.10 ppm

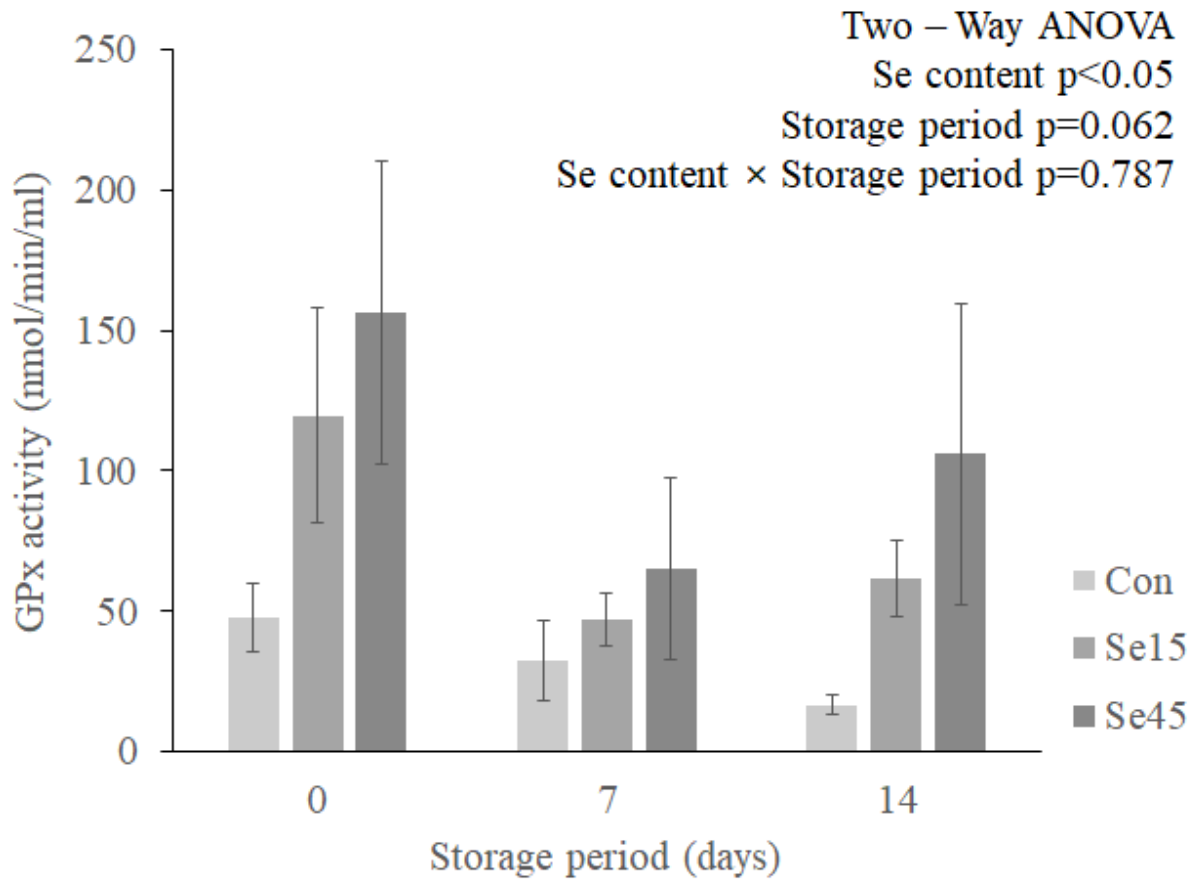
514 A-C Different letters in the same storage days indicate significant differences among selenium feeding  
515 conditions ( $P < 0.05$ ).

516 a-c Different letters within the same selenium feeding conditions indicate significant differences during  
517 storage ( $P < 0.05$ ).

518

519



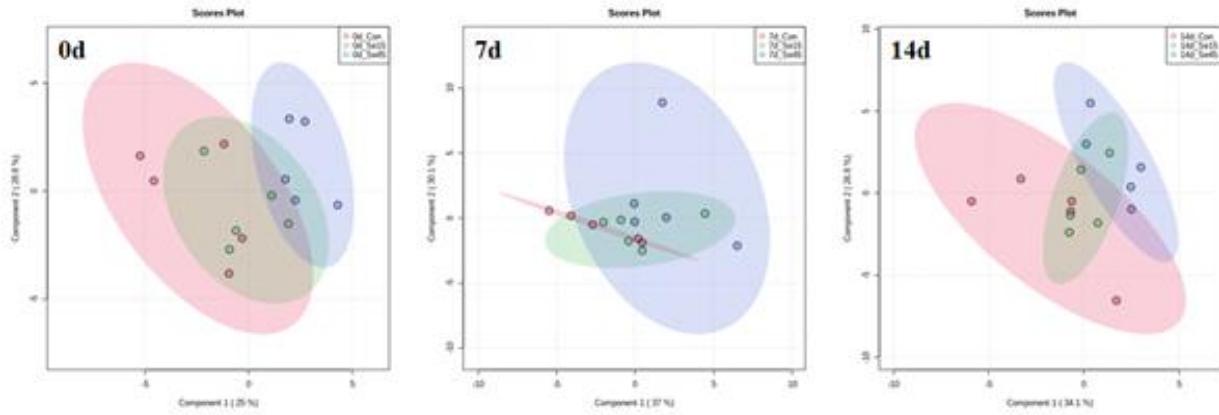


521

522 **Fig. 2.** Glutathione peroxidase (GPx) activity of pork loin raised under different selenium  
 523 supplementation conditions and storage period. Abbreviations: Se15, pork loin from feeding organic Se  
 524 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic Se 0.45 ppm + inorganic Se  
 525 0.10 ppm; GPx, Glutathione peroxidase

526

527



529

530

531

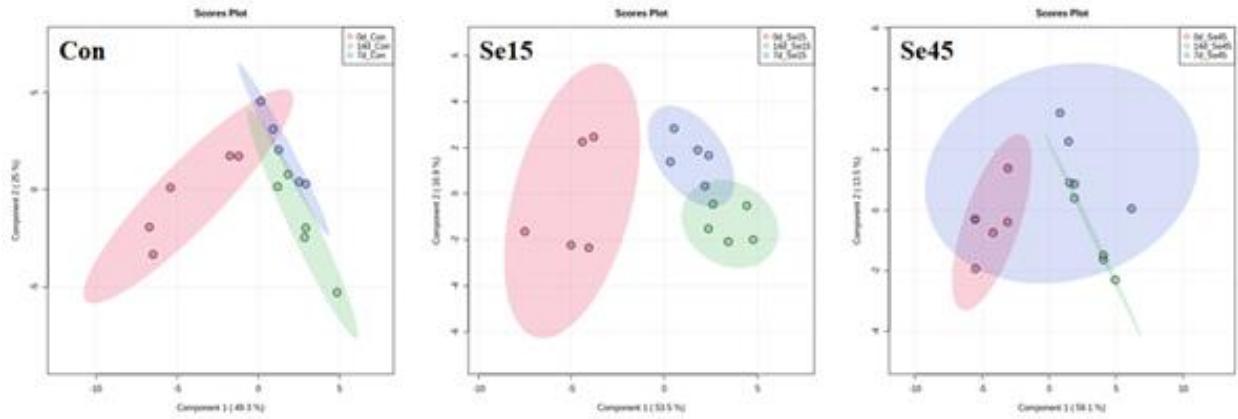
532

533

534

535

**Fig. 3.** Partial least squares-discriminant analysis of metabolites by storage period from pork loin raised under different selenium supplementation conditions and storage period. Abbreviations: Se15, pork loin from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic Se 0.45 ppm + inorganic Se 0.10 ppm



537

538 **Fig. 4.** Partial least squares-discriminant analysis of metabolites by treatment group from pork loin raised

539 under different selenium supplementation conditions and storage period. Abbreviations: Se15, pork loin

540 from feeding organic Se 0.15 ppm + inorganic Se 0.10 ppm; Se45, pork loin from feeding organic Se 0.45

541 ppm + inorganic Se 0.10 ppm

542

543

