Article

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Meat quality, free fatty acid concentration, and oxidative stability of pork from animals fed diets containing different sources of selenium

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Abstract

This study evaluates the effect of the source of dietary selenium supplementation (organic versus mineral) and the combined effect of organic selenium and vitamin E on the fatty acid composition and lipolysis in pork intramuscular fat and other meat quality characteristics such as drip loss and lipid stability. Higher vitamin E deposition, lower drip loss, and higher stability against oxidation were detected in muscle from pigs fed organic selenium. Also higher proportion of free fatty acids was observed in intramuscular fat from pigs fed organic selenium than those fed inorganic selenium, being these mainly coming from neutral lipid fraction. In addition, the inclusion of vitamin E in the diet enhanced such effect. Dietary organic selenium also increased Δ^9-desaturase and elongase indexes and C18:1n−9 concentration. A related decrease of C18:0 concentration was also observed. Described differences in the lipid fraction composition could affect sensory characteristics of meat and meat products.

Keywords

Organic selenium, vitamin E, drip loss, thiobarbituric acid reactive substances, lipolytic enzymes, free fatty acids

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INTRODUCTION

The inclusion of antioxidants, such as selenium (Se) and/or vitamin E, in animal diets is one of the most used dietary interventions focused in the improvement of meat quality. Some pork quality parameters improved by vitamin E are color, lipid stability against oxidation (Faustman and Want, 2000; Rey et al., 2004), and water holding capacity (WHC) (Ashgar et al., 1991; Monahan et al., 1992). Effects of dietary vitamin E supplementation, such as the reduction of cellular water losses, are explained by its antioxidant effect on cell membranes (Apple, 2007).

In animals, Se is necessary for selenoproteins, which play a key role in numerous metabolic processes such as the defense against free radicals and their detoxification (Lener et al., 2012) thereby, reducing the risk of many diseases (Fairweather-Tait et al., 2011). In humans, meat enriched with Se may improve body Se levels, especially in Se-deficient populations. Se has been added to swine diets as sodium selenite (Na_2SeO_3) and Se-methionine from Se-enriched yeasts, an inorganic and organic form, respectively. Higher antioxidant activity has been reported for the organic Se, whereas the inorganic form may act as a prooxidant (Spallholz, 1994) and have toxic effects particularly at high levels (Kim and Mahan, 2001;

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Seko et al., 1989). Organic Se has also higher effectiveness in delaying postmortem oxidation reactions (Mahan et al., 2014). The antioxidant functions of Se are related to the antioxidant enzyme glutathione peroxidase (Schrauzer, 2000) which has been shown their persistence in postmortem muscle tissue (Mahan et al., 2014). Moreover, when diets are supplemented with the organic form, higher amount of Se is accumulated in muscles and organs of pigs (Kim and Mahan, 2001). In addition, it has been reported that dietary Se also improves WHC and color, especially when it is supplied as organic form (Bobček et al., 2004; Mateo et al., 2007).

Some recent works indicate that the inclusion of antioxidants in animal diets can not only improve the oxidative stability of meat but also the activity of some enzymes. Particularly, it has been observed an enhanced postmortem activity of calpains by the inclusion of vitamin E (Rowe et al., 2004). Fully related is the proposition of Huff-Lonergan and Lonergan (2005) in which they describe that calpain activation may increase a rapid proteolysis of intermediate filament proteins in meat (mainly desmin), avoiding shrinking of the overall muscle cell and, consequently, reducing cell membrane contraction, directly linked to WHC. Supporting these results, Calvo et al. (2016) described that organic Se effectively increased WHC and postmortem muscle proteolytic activity. Additionally, it has been reported that Se may regulate adipogenesis and lipolysis in the organism (Wang et al., 2016) and that some vitamins may also have metabolic regulation of lipids in vivo (Koshkenbaev et al., 1985). However, there is no information on the possible lipolytic effects postmortem of the different forms of Se in the literature. Therefore, it was hypothesized that Se source may also affect the lipolysis process happening after slaughter in pigs.

The objective of the present research was to study the effect of the source of dietary Se supplementation in pigs (organic as Se-enriched yeast versus mineral as sodium selenite in feed) and the combined effect of organic Se and vitamin E on the fatty acid composition and, therefore, lipolysis in pork intramuscular fat. In addition, other meat quality characteristics such as drip loss or lipid stability were studied.

MATERIAL AND METHODS

All the experimental procedures used in this study comply with the Spanish guidelines for the care and use of animals in research (BOE, 2013).

Animals, experimental diets, and sample collection

Thirty pigs (Topigs 20 × Top York Topigs international) were randomly selected at an average live weight of 61.2 ± 0.5 kg and distributed into three groups (COPISO, Soria, Spain). Each pig was housed in an individual box during the experimental period. Diets were formulated to provide nutrient composition above NRC (2012) recommendation diets (Table 1) and were identical in composition except for the selenium source: sodium selenite (Na_2SeO_3) (SeS), Se-methionine from a Se-enriched yeast diet (*Saccharomyces cerevisiae*, SelPlex; Alltech, Spain; Commission Regulation (EC) No. 1750/2006) (SeY). A third group included organic Se and vitamin E (100 mg alpha-tocopheryl acetate per kg diet) (SeY-E). In all cases, Se was introduced in the vitamin–mineral mix to achieve a final concentration of $0.4\,mg\,kg^{-1}$ (Table 1). Pigs were fed the experimental diets until the end of the experiment (65 days in total), with 120.4 ± 2.5 kg as final body weight. Food and water were provided ad libitum during the duration of the study. At the end of the experiment period, pigs were sent to a commercial slaughterhouse (Incarlopsa, Tarancón, Spain) and slaughtered after a fasting period of 20 h. Carcasses were chilled (4 °C) and samples, approximately 50 cm in size, were taken from the longissimus thoracis (LT) muscle.

Methods

Drip loss in muscle samples. For the determination of weight loss during storage, approximately $1\,cm^3$ samples (weighing approximately 10 g) were taken from the LT muscle. After cutting, samples were weighed and placed under refrigerated conditions at 4 °C in a saturated atmosphere. Samples were weighed again at 72 h of storage. The difference between final and initial weights was used to calculate the drip loss that was expressed as a percentage of the initial weight (Honikel, 1997).

Instrumental color analysis. Two-centimeter-thick samples were placed on trays and kept at 4 °C for color measurement. Muscle color was evaluated on days 1 and 6 after slaughter by means of a Chroma Meter (CM 2002, Minolta, Camera, Osaka, Japan) previously calibrated against a white tile in accordance with the manufacturer's recommendations (CIE, 1976). The average of five random readings was used to measure lightness (L*), redness (a^*), and yellowness (b^*). The light source was set to D65 and the aperture to 10°.

Mineral determination. Digestion of muscle samples was carried out by nitric acid in a Teflon sealed pump. The quantification of Se was carried out in an atomic absorption spectrometer equipped with graphite chamber (GF-AAS9 UNICAM 939QZ). Ca, P, and Zn

Table 1. Ingredients and chemical composition of the experimental diets.

	SeS[1]	SeY[2]	SeY-E[3]
Ingredients[4](mg kg^{-1})			
Vitamin E (α-tocopherol)	21.6	22.4	121.1
Selenium (Na_2SeO_3)	0.4	0	0
Selenium (Selplex)	0	0.4	0.4
Chemical composition(g kg^{-1})			
Dry matter	8.98	8.98	8.98
Crude protein	1.32	1.32	1.32
Fat	0.62	0.62	0.62
Crude fiber	0.42	0.42	0.42
Ash	0.44	0.44	0.44
Starch	4.63	4.63	4.63
Lysine dig	0.07	0.07	0.07
Met dig	0.02	0.02	0.02
Met + Cys dig	0.04	0.04	0.04
Thr dig	0.04	0.04	0.04
Premix[5]	8.98	8.98	8.98
DE (kcal kg^{-1})	2449	2449	2449
Fatty acids (mg kg^{-1})			
C16:0	7.3	8.0	8.2
C18:0	2.6	3.0	3.2
C18:1 n−9	9.8	9.9	10.1
C18:1 n−7	0.6	0.5	0.7
C18:2 n−6	8.4	8.9	8.5
C18:3 n−3	0.8	0.7	0.9

[1]SeS: group of sodium selenite added.
[2]SeY: organic selenium group.
[3]SeY-E: organic selenium and vitamin E group.
[4]Ingredients (g kg^{-1}): barley, 484.4; wheat, 25.0; corn, 8.00; soya cake 47, 6.00; sunflower oil, 5.00; mixed fats, 4.50; calcium carbonate, 1.03; L-lysine, 0.54; sodium bicarbonate, 0.50; bicalcium phosphate, 0.37; salt, 0.26; L-threonine, 0.08; bactericide, 0.05; fungicide, 0.03.
[5]Premix (per kilogram of finished diet): vitamin A: 8,000,000 IU; vitamin D_3: 1,800,000 IU; vitamin B_1: 500 mg; vitamin B_2: 150 mg; vitamin B_{12}: 12 mg; vitamin B_6: 1000 mg; nicotinic acid: 1500 mg; calcium pantothenate: 5000 mg; vitamin K_3: 250 mg; choline chloride: 100,000; $FeCO_3$: 35,000 mg; $CuSO_4{\cdot}5H_2O$: 7500 mg; Co (hydrate carbonate): 25 mg; ZnO: 50,000 mg; Mn (oxide): 20,000 mg; KI: 250 mg; 3-fitase EC 1,6,3,2: 325,000 FTU; BHT E321: 1000 mg; citric acid E330: 3450; sodium citrate E331: 100 mg.

determination was done by optical emission spectroscopy with inductively coupled plasma (ICP-OES OPTIMA 3300DV). All the analyses were carried out in the research support center (Centro de apoyo a la investigación) of Geological Techniques of Complutense University of Madrid.

Tocopherol quantification. The α-tocopherol concentration in muscle samples was quantified by direct extraction as described by Rey et al. (2006). Thus, muscle samples were mixed with 0.054 M dibasic sodium phosphate buffer adjusted to pH 7.0 with HCl and absolute ethanol. After mixing, the tocopherol was extracted with hexane by centrifugation. The upper layer was evaporated to dryness and dissolved in ethanol prior to analysis. Tocopherols were analyzed by reverse phase HPLC (HP 1100, equipped with a diode array detector; Agilent Technologies, Waldbronn, Germany) as described elsewhere (Rey and López-Bote, 2014). Identification and quantification were carried out using a standard curve ($R^2 = 0.999$) of the pure compound (Sigma, Alcobendas, Madrid). All samples were analyzed in duplicate. The α-tocopherol concentration in muscle was assessed on day 0.

TBARS analysis. Oxidation was assessed on days 1 and 7 by the thiobarbituric acid method described by Salih et al. (1987). Twenty-seven milliliters of perchloric acid (3.83% v/v) was added to 5 g of meat and the mixture was homogenized with an Ultra-Turrax homogenizer for 1 min and filtered through filter paper. Aliquots were added to thiobarbituric acid (0.02 M) (1:1) and

heated in boiling water for 15 min. A standard curve was prepared with 1,1,3,3-tetraethoxypropane in water. Absorbance was measured at 532 nm and the values were expressed as milligram of malondialdehyde (MDA) per kilogram meat.

Glutathione quantification. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were quantified spectrophotometrically at 405 nm in deproteinized muscle using the corresponding diagnostic colorimetric kit (Arbor assays, USA). As kit requirements indicate, muscle samples were homogenized at 10 mg/250 µl in ice cold 5% 5-sulfo-salicylic acid dihydrate solution, incubated at 10 min at 4 °C, then centrifuged at 14,000 r/min for 10 min at 4 °C to remove precipitated protein and the supernatant was collected. The concentration of GSSG was determined from the 2-vinylpyridine-treated samples read off 2-vinylpyridine-treated standard curve. Concentrations of free glutathione (GSH-free) were obtained from the 2-vinylpyridine-treated standard from nontreated standards and samples (total GSH). The concentrations obtained were expressed as micromolar of glutathione per gram of meat.

Lipid fractions of intramuscular fat. Lipids from muscle samples were extracted and methylated using the procedure described by Segura and López-Bote (2014). Lyophilized samples (50 g frozen meat for 72 h at 25 °C and 0.2 bar; Lyoquest, Telstar, Terrassa, Spain) were accurately weighed in a safe-lock micro test tube, homogenized in 1.5 ml dichloromethane–methanol (8:2), and mixed in a mixer mill (MM400, Retsch technology, Haan, Germany). The final biphasic system was separated by centrifugation (8 min at 10,000 r/min) and the collected solvent was evaporated under nitrogen stream. The lipid content was gravimetrically determined. Total lipid extracts were fractionated in neutral lipids (NL), free fatty acids (FFA), and polar lipids (PL) using the procedure described by Ruiz et al. (2004). Briefly, 20 mg of intramuscular fat dissolved in 0.15 ml of hexane:chloroform:methanol (95:3:2) was added to a 500 mg aminopropyl minicolumn (Varian, CA, USA), which had been previously activated with 7.5 ml of hexane. NL were eluted with 5 ml of chloroform, FFA were eluted with 5 ml of diethylether:acetic acid (98:2) and PL were eluted in two different fractions. The first one with 2.5 ml methanol:chloroform (6:1) and the second one with 2.5 ml of sodium acetate in methanol:chloroform (6:1). The two fractions containing PL obtained with this method were mixed and analyzed together.

Fatty acid profile. Lipid fractions were subjected to esterification by heating the obtained fractions at 80 °C for 1 h in 3 ml of methanol:toluene:H_2SO_4 (88:10:2 by volume) as described by Garcés and Mancha (1993). After tempering, the fatty acid methyl esters (FAMEs) were extracted with hexane and directly injected in GC system.

Gas–liquid chromatography conditions. FAMEs were separated using a gas chromatograph (HP 6890 Series GC System) equipped with flame ionization detector. Separation was performed with a J&W GC Column, HP-Innowax Polyethylene Glycol (30 m × 0.316 mm × 0.25 µm). Nitrogen was used as a carrier gas. After injection at 170 °C, the oven temperature was raised to 210 °C at a rate 3.5 °C min^{-1}, then to 250 °C at a rate of 7 °C min^{-1} and held constant for 1 min. The flame ionization was held at 250 °C. FAME peaks were identified by comparing their retention times with those of authentic standards (Sigma–Aldrich, Alcobendas, Spain), quantified by using C15:0 as internal standard and expressed as percentage (%).

Unsaturation index was calculated as follows: 1 (% monoenoics)+2 (% dienoics)+3 (% trienoics)+4 (% tetraenoics) + 5 (% pentaenoics) + 6 (% hexaenoics). Average chain length was calculated as Σ (n × (% fatty acid) n)/100, n = number of atoms of carbon. Desaturase and elongase indexes were calculated according to Korniluk et al. (2008).

Statistical analysis. The experimental unit for analysis of all data was the pig. Data were analyzed following a completely randomized design using the general linear model procedure contained in SAS (SAS, 2009). Data were presented as the mean of each group and root mean square error together with significance levels (P-value). Duncan's test was used to separate treatment means. Means were statistically different when P-value was lower than 0.05.

RESULTS AND DISCUSSION

Tocopherol accumulation, selenium, mineral content, and oxidative status of muscle

Concentration of α-tocopherol (vitamin E) in muscle of pigs is presented in Table 2. Muscle samples from pigs fed SeY group showed a 23% increased α-tocopherol level than those fed SeS ($P < 0.0001$) even though same concentrations of vitamin E were added to the feed. There is a synergism between Se and vitamin E to protect against cellular damage caused by reactive oxygen species (Saito et al., 2003), which may enhance meat quality (Surai, 2002). It has been reported that absorption of vitamin E is impaired by Se deficiency, and dietary Se alleviates such deficiency, allowing higher levels of vitamin E to be absorbed (Finkel and

Table 2. Effect of dietary selenium source (organic, SeY versus mineral, SeS) and vitamin E and organic selenium (SeY-E) on α-tocopherol (μg g^{-1}), intramuscular fat (IMF, g kg^{-1}), drip loss (g kg^{-1}), water loss by lyophilization (g kg^{-1}), moisture (g kg^{-1}), minerals (ppm), TBARS (mg MDA kg^{-1} meat) on days 0, 3, and 6 of refrigerated storage, glutathione (μM g^{-1} meat) and color parameters (CIE L*, *a**, *b**) on days 0 and 6 of refrigerated storage in muscle samples from pigs fed the experimental diets.

	SeS	SeY	SeY-E	RMSE	P
Drip loss	30.6 a	26.9 ab	25.5 b	8.4	0.1049
Water loss (72 h lyophilization)	746 a	739 b	740 b	4.7	0.0001
Moisture	791	785	788	5.2	0.5884
IMF	48.8	49.1	49.3	1.5	0.7080
Vitamin E	1.7 c	2.1 b	3.1 a	0.70	0.0001
Se	0.2 b	0.3 a	0.3 a	0.07	0.0742
Ca	66 b	76 ab	80 a	13.8	0.0079
P	2074 b	2376 a	2305 a	132	0.0007
Zn	67 b	95 a	91 a	24.1	0.0178
TBARS					
Day 0	2.0	1.8	1.9	1.02	0.8092
Day 3	2.5 a	1.9 b	2.0 b	1.06	0.0713
Day 6	3.2 a	2.7 ab	2.3 b	1.06	0.0381
Muscle glutathione					
GSSG	0.9 a	0.6 b	0.4 c	0.14	0.0211
GSH free	37.0 a	33.3 b	32.1 b	7.97	0.0337
GSSG/GSH	2.6 a	1.5 b	1.3 b	0.01	0.0209
Color parameters					
L*					
Day 0	52.1	52.0	52.1	3.28	0.9874
Day 6	51.3	50.9	51.3	3.29	0.9063
*a**					
Day 0	2.1 b	2.6 a	2.3 ab	0.79	0.1082
Day 6	1.7	2.0	1.7	0.83	0.2786
*b**					
Day 0	11.9	12.4	12.2	1.14	0.2822
Day 6	11.9	12.1	12.0	0.96	0.7405

GSH: free glutathione; GSSG: oxidized glutathione; IMF: intramuscular fat; RMSE: root of the mean squares error from the main effect; TBARS: thiobarbituric acid reactive substances.
Different letter in the same row indicates statistical difference ($P < 0.05$) between dietary treatments.

Holbrook, 2000). However, there is not much available information on the possible effects of the Se source on muscle vitamin E accumulation. Calvo et al. (2017) described a higher vitamin E deposition in LT muscle of pigs fed with Se supplemented diets, independently of the Se source (organic or inorganic) and concentration (0.2 and 0.4 ppm), when compared to a control group (0.0004 ppm Se). Besides, the results of the present study agree with those found by Calvo et al. (2017) who also found a trend toward a higher vitamin E muscle concentration in groups supplemented with the organic Se form ($P = 0.08$) when compared to those fed inorganic source, similarly as observed in the present study (Table 2).

Higher Se accumulation in muscle was observed in pigs fed SeY and SeY-E when compared to SeS (0.3 versus 0.2 ppm, respectively, $P = 0.0742$) (Table 2). Some studies using a Se supplementation of 0.3 ppm in the diet showed higher effectiveness in the accumulation of organic than inorganic Se in pig muscle (Jang et al., 2010; Mahan et al., 2014). Organic Se (in the form of Se-enriched yeast) is taken up via methionine transporter mechanisms and can be incorporated into selenoenzymes or in place of methionine in general body proteins (Suzuki and Ogra, 2002).

Attending to other mineral concentrations in the present study, Ca, Zn, and P concentrations were higher in pigs fed with SeY and SeY-E ($P < 0.01$)

when compared to those fed SeS. Krajewska et al. (2012) found higher Ca in pancreas and higher Zn concentration in spleen of lambs fed with selenate (Se VI) when compared to a control group.

The evaluation of the oxidative status of the pig meat according to the sources of selenium supplementation (TBARS) is also shown in Table 2. The group supplemented with SeS tended to have higher MDA concentration in muscle samples than the SeY group ($P = 0.0713$) after day 3 of refrigerated storage. At day 6, MDA concentration was numerically lower in SeY than in SeS; however, differences were only statistically significant when comparing SeS and SeY-E groups whereas SeY group showed intermediate values. Li et al. (2011) and Zhan et al. (2007) reported higher stability of muscle samples against lipid oxidation by SeY in pork. Similar results were observed in meat from male broilers (Chen et al., 2014) and female turkeys (Mikulski et al., 2009). Se-methionine is the main form of organic Se present in Se-enriched yeast. Such compound is an efficient scavenger of peroxynitrite, a product of nitric oxide and superoxide anion and a strong oxidant capable of reacting with a high variety of biomolecules (Padmaja et al., 1996). Concerning the combination of SeY and vitamin E, a lack of effect of vitamin E on MDA production when compared to SeY group was observed, which could be due to the supplementation dose used in the present study ($100\,mg\,kg^{-1}$). Authors who reported more efficient effect of dietary vitamin E supplementation on lipid oxidation (Faustmand and Want, 2000; Rey et al., 2004) used higher vitamin E dose ($200\,mg\,kg^{-1}$). However, others (Kawecka et al., 2013) who used low-supplementation dose ($60\,mg\,kg^{-1}$) did not find beneficial effect of vitamin E and organic Se on the quality of meat. In a previous study carried out by our group (Calvo et al., 2016), using a supplemented diet with inorganic Se and $100\,mg\,kg^{-1}$ of vitamin E, a reduction in TBARS numbers was found when compared to a control group; however, muscle vitamin E accumulation was higher than that reported in the present study and differences between low-supplemented and high-vitamin E supplemented groups were of higher magnitude than those found in the present study for groups SeY and SeY-E.

In order to confirm the effects of the dietary treatments on the oxidative stability, the measurement of oxidized-to-reduced glutathione ratio (GSSG/GSH) was carried out (Table 2). Glutathione reductase is an enzyme related to redox homeostasis. Upon absorption of inorganic Se compounds, the higher valence forms (selenite (Se(IV))) are reduced to the selenide state using reducing equivalents from GSH reductase and NADPH, and the organic forms release Se in the selenide state as a result of catabolism (Finley, 2006). Together, Grant et al. (1996) described that increased glutathione reductase activity under oxidative stress produces higher GSH concentration and Salami et al. (2016) supported that GSSG/GSH ratio increases under oxidative conditions. In Table 2, it can be observed that the SeY-E group showed the lowest GSSG concentration and SeS the highest, whereas SeY had an intermediate value (0.4, 0.9, and $0.6\,\mu M\,g^{-1}$ respectively, $P < 0.05$). Similarly, lower GSSG/GSH ratio for both the SeY and SeY-E groups versus the SeS one was observed. These results would confirm a better antioxidant muscle status in groups supplemented with the organic form of Se.

Drip and water losses of muscle

Concerning the effects of dietary Se forms on muscle drip loss, in the present study, only a tendency ($P = 0.1$) to higher drip loss values was observed in SeS group when compared to the others. Nevertheless, a significant higher water loss obtained by lyophilization was found in the SeS group ($P < 0.0001$, Table 2). There is information in the literature that reports higher WHC value in pigs fed the organic Se (Calvo et al., 2016; Li et al., 2011; Lisiak et al., 2014; Mahan et al., 1999; Zhan et al., 2007). Whereas, others observed no effect (Castro-Ríos and Narváez-Solarte, 2013). The departing hypothesis of this work was the fact that a higher effectiveness of Se-dependent enzymes also increases the cell integrity and/or reduces drip channels. Such assumptions are based on previous studies. Huff-Longergan and Lonergan (2005) described that proteolysis of key cytoskeletal proteins as desmin may be related to drip production. These proteins have been shown to be degraded as early as 45 min to 6 h postmortem in some muscles. Degradation of these proteins at early postmortem time would certainly allow water, which would be expelled from the intramyofibrillar spaces, to remain in the cell for a longer period or, as Farouk et al. (2012) explained, to avoid the formation of drip channels. The lysis of such proteins is mediated by calpain activity. Hence, higher degradation of commented proteins must imply an increase of calpain activity and activation. If intermediate filament proteins would be more effectively broken, higher protein fragment concentration will be found in the sarcoplasm and therefore, less drip loss will be expected.Supporting such information, the lyophilization process is based on the conversion of water from solid to gas state and therefore, dependent on the structure through water molecules have to defund. In muscle system, water is linked to several structures. These facts would support the different behavior in water migration by lyophilization process observed in Table 2, which directly indicates that dietary Se source may affect water linkage to

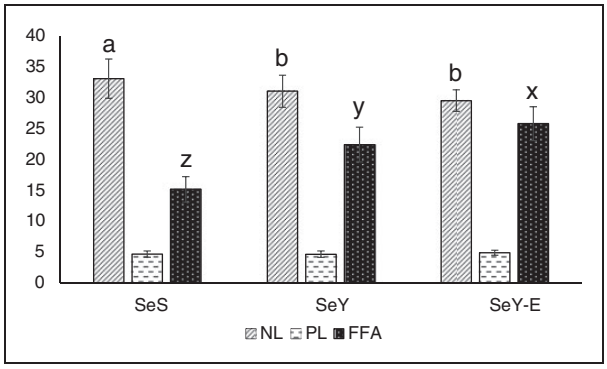

Figure 1. Concentration (milligram per gram extracted lipids) of neutral lipids (NL), free fatty acids (FFA), and phospholipids (PL) present in longissimus thoracis muscle from pigs fed the experimental diets: 0.4 ppm of inorganic selenium and 21.6 ppm of vitamin E (SeS), 0.4 ppm of organic selenium and 22.4 ppm of vitamin E (SeY), and 0.4 ppm of organic selenium and 121.1 ppm of vitamin E (SeY-E). Different letter indicates statistical difference ($P < 0.05$) between dietary groups and same lipid fraction.

biomolecules. Besides, both the results and the considered hypothesis are in agreement with the results reported by Calvo et al. (2016) who found higher WHC and higher proteolytic activity when organic Se was supplied.

Instrumental color

Instrumental color parameters are presented in Table 2. No color changes, measured as CIE L* (lightness), *a** (red color), *b** (yellow-green color), regarding to storage time were observed. CIE L* and *b** parameters were not affected by dietary treatment in the present study. However, SeS-enriched pigs showed lower *a** value in muscle than those from SeY group. Zhan et al. (2007) described higher *a** values for loin samples of pigs fed with a Se-enriched diet than control ones and an increase of such difference with time when organic Se was used. Besides, Mahan et al. (1999) observed paler muscle tissue in pigs fed with inorganic

Table 3. Fatty acid profile (g fatty acid per 100 g of quantified fatty acid) of neutral lipids fraction of longissimus thoracis muscle from pigs fed the experimental diets: 0.4 ppm of inorganic selenium and 21.6 ppm of vitamin E (SeS), 0.4 ppm of organic selenium and 22.4 ppm of vitamin E (SeY), and 0.4 ppm of organic selenium and 121.1 ppm of vitamin E (SeY-E).

	SeS	SeY	SeY-E	RMSE	P
C16:0	25.4	24.7	24.9	1.156	0.3858
C16:1 n−7	3.89	4.29	3.88	0.397	0.1656
C18:0	14.8 a	13.4 ab	12.5 b	0.689	0.0012
C18:1 n−9	48.1 b	50.5 a	50.0 a	1.315	0.0297
C18:2 n−6	5.54 b	4.88 b	6.27 a	0.653	0.0016
C18:3 n−3	0.45	0.45	0.51	0.088	0.3156
C20:0	0.24	0.25	0.24	0.029	0.9319
C20:1 n−9	0.92	0.92	0.94	0.082	0.8481
C20:3 n−6	0.62 b	0.59 b	0.83 a	0.144	0.0072
C20:4 n−6	0.06	0.06	0.06	0.013	0.7844
ΣSAFA	40.4 a	38.4 b	37.6 b	1.546	0.0212
ΣMUFA	52.9 b	55.7 a	54.8 a	0.897	0.0439
ΣPUFA	6.66 b	5.97 b	7.68 a	0.738	0.0008
UI	67.4 b	68.8 ab	71.6 a	1.483	0.0031
ACL	17.5	17.5	17.5	0.143	0.1696
Σn−6/Σn−3[1]	14.0	12.2	14.1	3.808	0.1186
Δ^5-desaturase[2]	0.09	0.09	0.07	0.032	0.3614
Δ^9-desaturase[3]	0.56 b	0.59 a	0.59 a	0.021	0.0033
Elongase[4]	0.18	0.16	0.15	0.081	0.6527

ACL: average chain length; ΣMUFA: total of monounsaturated fatty acids; ΣPUFA: total of polyunsaturated fatty acids; RMSE: root of the mean squares error from the main effect; ΣSAFA: total of saturated fatty acids; UI: unsaturation index.
[1]Σn−6/Σn−3: (C18:2 n−6 + C20:3 n−6 + C20:4 n−6)/C18:3 n−3.
[2]Δ^5-desaturase: C20:4 n−6/(C20:3 n−6 + C20:4 n−6).
[3]Δ^9-desaturase: (C16:1 n−7 + C18:1 n−9)/(C16:0 + C16:1 n−7 + C18:0 + C18:1 n−9).
[4]Elongase: (C18:0 + C20:1 n−9)/(C16:0 + C18:0 + C18:1 n−9 + C20:1 n−9).
Different letter in the same row indicates statistical difference ($P < 0.05$) between dietary treatments.

Se diet when compared to those fed organic Se. Accordingly, Calvo et al. (2017) found similar differences in color parameters attending to Se source.

Fatty acid profile

The concentration of FFA, NL, and PL depending on the treatment is shown in Figure 1. As it can be observed, the highest concentration of FFA was found in samples from SeY-E diet and the lowest in the SeS one, whereas SeY showed intermediate concentration. Such increase of FFA seems to be partially associated with a depletion of NL concentration while PL remained constant. These results suggest that dietary antioxidants administration produces higher postmortem lipolysis in pork, thus leading to a higher FFA concentration. This is in agreement with previous finding in which antioxidants administration protected proteolytic enzyme integrity (Calvo et al., 2016). It can be speculated that such antioxidant status may also affect postmortem lipolytic enzyme activity, leading to higher FFA concentration. Nevertheless, in addition to the stabilizing enzymatic effect mainly due to the antioxidant Se properties, another possible explanation could be found in the insulin resistance effects related to the supranutritional Se supply. Dimitriadis et al. (2011) reviewed the insulin effects in muscle and adipose tissue and stated a decrease of lipolysis and an increase of triacylglycerol and fatty acid synthesis as a consequence of a promoted glucose uptake. Stapleton (2000) discussed the available evidence in support of Se as an insulin mimetic. Subsequently, Pinto et al. (2012) and Zeng et al. (2012) explained, in pigs and rats, respectively, that the inclusion of a supranutritional concentration of Se in the diets would induce an insulin resistance thus increasing lipolysis among other metabolic disorders. In fact, it has been found that Se in its selenite form may alter the expression of genes involved in different lipid metabolic pathways including lipolysis activity (Hassan et al., 2014). Furthermore, Wang et al. (2016) recently reported that the biological effect of Se on metabolic function of mice high-fat diet-induced

Table 4. Fatty acid profile (g fatty acid per 100 g of quantified fatty acid) of the free fatty acid fraction of longissimus thoracis muscle from pigs fed the experimental diets: 0.4 ppm of inorganic selenium and 21.6 ppm of vitamin E (SeS), 0.4 ppm of organic selenium and 22.4 ppm of vitamin E (SeY), and 0.4 ppm of organic selenium and 121.1 ppm of vitamin E (SeY-E).

	SeS	SeY	SeY-E	RMSE	P
C16:0	21.4	22.1	21.4	1.865	0.4829
C16:1 n−7	2.89	3.12	2.74	0.585	0.2203
C18:0	19.4 a	16.6 b	16.8 b	2.727	0.0143
C18:1 n−9	32.0	33.4	34.4	2.988	0.0943
C18:2 n−6	15.9	16.8	16.7	1.087	0.1027
C18:3 n−3	0.71	0.77	0.74	0.160	0.7622
C20:1 n−9	0.81	1.20	0.95	0.611	0.3705
C20:3 n−6	0.83	0.86	1.15	0.568	0.2649
C20:4 n−6	5.22	5.14	5.33	1.235	0.9158
ΣSAFA	41.4	39.9	39.6	4.435	0.4925
ΣMUFA	37.9	37.3	35.5	3.529	0.1785
ΣPUFA	20.7 b	22.9 ab	24.9 a	3.818	0.0181
UI	92.4	93.9	95.4	10.55	0.7526
ACL	17.6	17.6	17.6	0.084	0.4829
Σn−6/Σn−3[1]	30.9	29.9	30.3	4.631	0.8892
Δ^5-desaturase[2]	0.87	0.85	0.87	0.043	0.3557
Δ^9-desaturase[3]	0.46 b	0.49 a	0.49 a	0.022	0.042
Elongase[4]	0.45 b	0.49 a	0.50 a	0.038	0.0113

ACL: average chain length; ΣMUFA: total of monounsaturated fatty acids; ΣPUFA: total of polyunsaturated fatty acids; RMSE: root of the mean squares error from the main effect; ΣSAFA: total of saturated fatty acids; UI: unsaturation index.
[1]Σn−6/Σn−3: (C18:2 n−6 + C20:3 n−6 + C20:4 n−6)/C18:3 n−3.
[2]Δ^5-desaturase: C20:4 n−6/(C20:3 n−6 + C20:4 n−6).
[3]Δ^9-desaturase: (C16:1 n−7 + C18:1 n−9)/(C16:0 + C16:1 n−7 + C18:0 + C18:1 n−9).
[4]Elongase: (C18:0 + C20:1 n−9)/(C16:0 + C18:0 + C18:1 n−9 + C20:1 n−9).
Different letter in the same row indicates statistical difference ($P < 0.05$) between dietary treatments.

insulin resistance was contradictory: posttreatment with Se promoted lipolysis in adipose tissue, whereas pretreatment with Se increased adipocyte differentiation and fat deposits in adipose tissue. These authors explained that activation of GPx1 was in part responsible for Se-exhibited bidirectional significance. These activation differences may also be responsible for differences in Se form effects of the present study; however, more investigations are needed.

It is also of interest to observe the combined effect of organic Se and vitamin E (SeY-E) on FFA and, indirectly, on lipolysis of muscle. This dietary treatment resulted in the highest concentration of FFA when compared to the other groups. Conversely to the effects described above, Koshkenbaev et al. (1985) reported that protein and vitamin insufficiency led to increase in the FFA levels. However, other studies reported that dietary vitamin E enrichment might enhance lipase activity (Kenari and Naderi, 2016). The results of the present study would indicate the possible potential lipolytic effect of dietary vitamin E, which combined with organic Se in diets, might enhance the observed Se effects on muscle postmortem lipolysis. Differences in lipase activity, and consequently in lipid fractionation, would result in different concentration of flavor precursors and differences in flavor development (Toldrá and Flores, 1998).

At this point, it is necessary to indicate that the observed increase in FFA concentration could improve the generation of flavor changes and then, the production of meat products with different sensory characteristics. During thermal lipid degradation, not all lipids contribute the same to aroma formation. Unsaturated fatty acids are more reactive during heating, thus

Table 5. Fatty acid profile (g fatty acid per 100 g of quantified fatty acid) of the polar lipid fraction of longissimus thoracis muscle of pigs fed with the experimental diets: 0.4 ppm of inorganic selenium and 21.6 ppm of vitamin E (SeS), 0.4 ppm of organic selenium and 22.4 ppm of vitamin E (SeY), and 0.4 ppm of organic selenium and 121.1 ppm of vitamin E (SeY-E).

	SeS	SeY	SeY-E	RMSE	P
C16:0	22.5	22.5	22.2	1.118	0.6792
C16:1 n−7	1.69 a	1.50 ab	1.44 b	0.121	0.0532
C18:0	8.78 a	8.12 b	8.05 b	0.786	0.0387
C18:1 n−9	21.2	20.2	20.7	2.251	0.5947
C18:2 n−6	29.8 b	31.4 a	31.3 a	1.928	0.0292
C18:3 n−3	0.78 a	0.60 b	0.60 b	0.093	0.0721
C20:3 n−6	0.50	0.50	0.58	0.124	0.1001
C20:4 n−6	10.3	10.8	10.7	1.359	0.4847
C20:5 n−3	0.48	0.49	0.52	0.072	0.2561
C22:4 n−6	1.72	1.62	1.56	0.229	0.1889
C22:5 n−3	1.60	1.56	1.70	0.202	0.1450
C22:6 n−3	0.70	0.64	0.66	0.155	0.6152
ΣSAFA	31.3 a	30.7 b	30.2 b	1.176	0.0370
ΣMUFA	22.9	21.7	22.1	2.419	0.3742
ΣPUFA	45.9	47.6	47.7	2.864	0.1061
UI	159	161	162	5.535	0.2240
ACL	17.9	17.9	17.9	0.052	0.4314
Σn−6/Σn−3[1]	11.9 b	13.5 a	12.7 ab	1.198	0.0077
Δ^4-desaturase[2]	0.30	0.29	0.28	0.038	0.2406
Δ^5-desaturase[3]	0.95	0.96	0.95	0.012	0.1758
Δ^9-desaturase[4]	3.16 a	2.82 b	2.81 b	0.338	0.0088
Elongase[5]	0.31	0.30	0.30	0.020	0.2731

ACL: average chain length; ΣMUFA: total of monounsaturated fatty acids; ΣPUFA: total of polyunsaturated fatty acids; RMSE: root of the mean squares error from the main effect; ΣSAFA: total of saturated fatty acids; UI: unsaturation index.
[1]Σn−6/Σn−3: (C18:2 n−6 + C20:3 n−6 + C20:4 n−6 + C22:4 n−6)/(C18:3 n−3 + C20:5 n−3 + C22:5 n−3 + C22:6 n−3).
[2]Δ^4-desaturase: C22:6 n−3/(C22:5 n−3 + C22:6 n−3).
[3]Δ^5-desaturase: C20:4 n−6/(C20:3 n−6 + C20:4 n−6).
[4]Δ^9-desaturase: (C16:1 n−7 + C18:1 n−9)/(C16:0 + C16:1 n−7 + C18:0 + C18:1 n−9).
[5]Elongase: (C18:0 + C20:5 n−3)/(C16:0 + C18:0 + C20:5 n−3 + C22:5 n−3).
Different letter in the same row indicates statistical difference ($P < 0.05$) between dietary treatments.

producing large quantities of volatiles (Wood et al., 2003). In addition, the contribution of individual fatty acids (C18:3 n−3, C18:2 n−6, and C18:1 n−9) to odor in cooked meat was shown to produce different cooked meat aroma profiles although fishy notes were related to the presence of C18:3 n−3 and exacerbated by the presence of ferrous iron used to catalyze the oxidative reactions (Campo et al., 2003).

Tables 3 to 5 show the fatty acid profile of NL, FFA, and PL fractions of lipids of LT muscle from pigs fed the experimental diets. In the present study, lower C18:0 concentration was found in the fatty acid profile of the three fatty acid fractions (NL, FFA, and PL, Tables 3 to 5) in SeY and SeY-E groups compared to SeS. Moreover, NL and FFA of SeY groups also showed higher Δ^9-desaturase index than group SeS ($P < 0.005$ and $P < 0.05$, respectively) that resulted in a higher proportion of C18:1 n−9 ($P < 0.05$) and MUFA ($P < 0.05$) in NL fraction in group SeY (Tables 3 and 4). However, in PL, the behavior in Δ^9-desaturase index was opposite and the proportion of C18:2 n−6 was higher ($P < 0.05$) in groups fed SeY. It is also of interest to observe that elongase index value in FFA increased when the SeY diet was used. Little information and some controversy exist about the effect of Se on fatty acid profile of pork. At odds, Nuernberg et al. (2002) established lower C18:3n−3 concentration in muscle microsomes and higher C18:0 and C18:2 n−6 concentrations in muscle mitochondria from pigs fed Se-enriched diets than those fed a control one. Nevertheless, and in agreement with the depicted results, Sang-Keun et al. (2009) found lower C18:3 n−3, C18:0, and C16:0 and higher C16:1 n−7 and C18:1 n−9 concentrations in loin from pigs fed a diet enriched with 0.3% chrysanthemum powder, 0.1% probiotics and 0.1% Se. These results also agree with those reported by Korniluk et al. (2008) in lambs who depicted a possible increase of Δ^9-desaturase when 0.2 ppm of inorganic Se were added, whereas Czauderna et al. (2010) showed the opposite effect when the dose was increased to 0.5 ppm. However, the combination of dietary high dose-vitamin E supplementation and SeY did not result in different monounsaturated or saturated fatty acid profile, whereas the proportion of PUFA, C18:2 n−6, and C20:3 n−6 increased when compared to the other groups. Already in the work of Nuernberg et al. (2002), authors affirmed that more research was needed to analyze the increase of PUFA concentration and the oxidative stability of muscular mitochondria from animals fed a diet enriched with vitamin E. Infante (1986) proposed that vitamin E and Se play a role in the desaturation of n−3 and n−6 PUFAs by participating in the microsomal electron transport chain and in a proposed peroxidase moiety of the desaturase complex, respectively. On the contrary, other authors explained that these positive effects on desaturase and elongase enzyme activities were due to its antioxidant properties (Rey et al., 2014).

CONCLUSIONS

The inclusion of 0.4 ppm of organic Se in pig diets enhances the tissue accumulation of Se in muscle and thus, the WHC (measured as drip loss and lyophilization losses). Organic Se also promotes a higher accumulation of tissue vitamin E when the same doses were supplied; however, this change in such antioxidant accumulation only resulted in a slight increased protection against oxidation.

Postmortem lipase activity increases when organic Se is included in the diet and most FFA came from the lipolysis of NL. The combination of the natural form of Se with vitamin E in the diet seems to increase this effect. Organic Se also enhances the activity of Δ^9-desaturase enzymes thus increasing the concentration of C18:1 n−9 with a concomitant decrease of C18:0 concentration. Consequently, meat from pigs fed organic Se could be considered of higher quality from the nutritional point of view and it has more beneficial for the health of consumers. Differences in the lipid fraction composition might also result in products of different sensory characteristics; however, more research is needed.

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