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Effect of dietary organic selenium on muscle proteolytic activity and water-holding capacity in pork

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ABSTRACT: This study evaluates the effect of dietary selenium (**Se**) supplementation source (organic, Se-enriched yeast; **SY** vs. inorganic, sodium selenite; **SS**), dose (0.2: **L** vs. 0.4: **H** mg/kg) and the combination of Se and vitamin E (**VITE+SS**) for 26 days on drip loss, TBARS, colour changes, myofibrillar protein pattern and proteolysis in pork. The lowest water losses were observed in the SY-H group when compared to the others. SY-H and VITE+SS groups presented lower myofibrillar protein hydrolysis/oxidation. VITE+SS supplementation also resulted in higher PRO, TRP and PHE content at days 2 and 7, whereas the SY group showed increased GLY and CAR and tended to have higher TAU and ANS at day 2. The myofibrillar fragmentation index was not modified by the dietary treatment; however, at day 8, it tended to be higher in groups supplemented with SeY and VITE+SS. The results of the present study might indicate a possible relation between muscle proteolysis and water loss.

Key words: organic selenium; vitamin E; drip loss; TBARS; myofibrillar proteins; free-amino acids

1. INTRODUCTION

Pork quality depends on important attributes such as water-holding capacity, colour and oxidative stability, which are decisive in terms of suitability for processing and storage. Unacceptable colour, water retention or stability of the fresh product are mainly important for their influence on consumer behaviour, but can also cause high economic losses in the processing industry.

Feeding strategies are widely used in animals to improve meat quality (Andersen, Oksbjerg, Young & Therkildsen, 2005). Many interventions have focused on dietary supplementation with antioxidants such as selenium (**Se**) and/or vitamin E. Hence, vitamin E fed above dietary requirements has been reported to protect against lipid oxidation (Buckley, Morrissey & Gray, 1995) and to successfully improve the colour stability of fresh meat (Faustman & Wang, 2000) and other meat quality characteristics such as water-holding capacity (Ashgar et al., 1991). This reduction in meat drip losses has been attributed to the antioxidant activity of vitamin E, which may stabilise membrane integrity post-mortem, thus retaining sarcoplasmic protein in cells (Ashgar et al., 1991).

Dietary Se supplementation has also been reported to have antioxidant activities and to be effective in delaying post-mortem oxidation reactions, especially the organic form (Mahan et al., 2014). It has been suggested that organic selenium is more effective because it reaches tissues more efficiently (Mahan, Cline & Richert, 1999; Jang et al., 2010; Mahan et al., 2014) and consequently pork shows improved water-holding capacity and colour (Mahan et al., 1999; Zhan, Wang, Zhao, Li & Zu, 2007). However, there are no evident effects of selenium on membrane stability, and the mechanism by which Se acts, especially the organic form, remains unclear. Furthermore, selenite at high doses has been reported to act as a pro-oxidant (Shen, Yang, Liu & Ong, 2000). 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 & Ogra, 2002). The incorporation of selenomethionine (Se-Met) into proteins is non-specific and directly related to the dietary intake of selenium/Se-Met, whereas the incorporation of selenium into selenocysteine is specific and mediated at the ribosomal level (Schrauzer, 2000). As a rule, the replacement of Met by Se-Met does not significantly alter protein structure but may influence the activity of enzymes if Se-Met replaces Met in the vicinity of the active site (Schrauzer, 2000). Hence,

it has been described that the Se-Met-substituted thymidylate synthase of E.coli exhibited a 40% higher specific activity than the normal enzyme (Boles et al., 1991).

In contrast to the general belief that drip loss is reduced by the protective effect of some antioxidants on the muscle membrane, Lonergan and Lonergan (2005) have proposed that proteolysis and even protein oxidation are key in influencing the moisture retention capacity of meat. Differences between animals and/or muscles in the oxidation of myofibrillar proteins and hence in the antioxidant defence system would influence calpain activity post-mortem (Lonergan & Lonergan, 2005; Rowe, Maddock, Lonergan & Huff-Lonergan, 2004). Since calpain enzymes contain histidine and SH-containing cysteine residues at their active sites, they are particularly susceptible to inactivation by oxidation (Lonergan & Lonergan, 2005). Calpain activation produces a rapid fragmentation of intermediate protein filaments in meat (such as desmin, which links myofibrils to the cell membrane), preventing shrinking of the overall muscle cell membrane (Lonergan & Lonergan, 2005), and consequently reducing drip loss (Melody et al., 2004). Moreover, desmin not only links myofibrils to cell membrane but it also connects the adjacent myofibrils which are key to transfer the shrinkage of myofibrils and maintain the integral structure of muscles (Lonergan & Lonergan, 2005). However, protein degradation not always increase water holding capacity of pork (Lawson, 2004; Zhang et al., 2006).

Some previous studies have shown that dietary supplementation with antioxidants such as vitamin E influence proteolysis in meat (Rowe et al., 2004); however, to the best of our knowledge no information exists on the possible effects of dietary Se on post-mortem protein degradation.

We hypothesise that in pigs, dietary organic Se may exert a different effect on myofibrillar protein hydrolysis/oxidation and proteolysis than the inorganic form or vitamin E dietary enrichment. The objectives of the present research were to study the effect of the selenium source (organic as Se-enriched yeast vs. mineral as sodium selenite in feed) and dose (0.2 vs. 0.4 mg/kg) on muscle proteolysis, myofibrillar proteins and other meat quality characteristics such as drip loss, TBARS, colour changes and pigment oxidation.

2. MATERIAL AND METHODS

All experimental procedures performed in this study complied with Spanish guidelines for the care and use of animals in research (BOE, 2013) and were in accordance with the protocols approved by the Complutense University of Madrid.

2.1. Animals, Experimental Diets and Sample Collection

One hundred and eighty halothane-negative Large white x Landrage female pigs (Topigs 20 x L337) were randomly selected at an average live weight of 61.3 ± 0.5 kg, distributed into six groups and housed in an environmentally controlled, slatted-floor facility (Centenera del Campo, Soria, Spain). Pigs (n=10) were housed in an individual box during the experimental period; hence, each treatment was replicated three times. Diets were formulated to provide a nutrient composition above NRC (2012) recommendations (Table 1) and were identical in composition except for the selenium source: sodium selenite (Na_2SeO_3) (SS) or selenomethionine from a Se-enriched yeast diet (*Saccharomices cervisae*, Sel-Plex; Alltech, Spain; Commission Regulation (EC) Nº 1750/2006) (SY). In all cases, Se was added to the vitamin mineral mix to achieve a final concentration of either 0.2 mg/kg (**SY-L** or **SS-L**) or 0.4 mg/kg (**SY-H** or **SS-H**) (Table 1). In addition, a positive control group was supplemented with vitamin E in the diet (100 mg dl-α-tocopheryl acetate/kg) (**VITE-SS**) and a negative control group received a basal level of vitamin E (15 mg/kg) but no selenium supplementation (**CONTROL**). Pigs were fed the experimental diets until the end of the experiment (26 days in total), with 120.4 ± 2.5 kg. Food and water were provided *ad libitum* for the duration of the study.

At the end of the experimental period, pigs were sent to a commercial slaughterhouse (Incarlopsa, Tarancón, Cuenca, Spain) stunned with CO_2 and slaughtered after a fasting period of 24 h. Then, carcasses were eviscerated and split down the centre of the vertebral column before being suspended in the air and chilled at 4°C (1m/s; 90% relative humidity) for 2 h. Next, 24 h after slaughter, electrical conductivity and pH were measured by means of a LFStar conductivity meter (Mattahäus Ingenieurbüro, Klausa, DE) and a portable pH meter pH*K21 (NWK Binar, Puergen, DE), respectively. Before pH measurement, the instrument was calibrated with pH 7.0 ± 0.02 and 4.0 ± 0.02 buffers. Once carcasses had been jointed, the untrimmed hams, shoulders and loins were kept in a chilled room at 4°C for 24 h and then weighed. Samples of approximately 15 cm in size were taken

from the *longissimus lumborum* muscle at the level of the last rib and were stored in individual, vacuum-packed plastic bags at -20°C until analysis.

2.2. Laboratory Analysis

2.1.1 Moisture Determination in Muscle Samples (Standard ISO-1442).

Muscle samples (5 g) were maintained in a dry heater at 100-105°C until constant weight. Humidity was determined gravimetrically at day 0 and after 8 days of refrigerated storage.

2.2.2 Drip Loss in Muscle Samples.

To determine weight loss during storage, approximately 1 cm^3 of sample (weighing approximately 10 g) was taken from the *longissimus lumborum* muscle. After cutting, samples were weighed, put inside of a mesh and a plastic bag that was closed and placed under refrigerated conditions at 4°C in a saturated atmosphere. Samples were weighed again after 72 hours of storage. The difference between initial and final weights was used to calculate drip loss, which was expressed as a percentage of the initial weight (Honikel, 1997). Frozen samples were previously thawed before following the same procedure as described above.

Weight loss was also gravimetrically quantified by sequenced weights of 2 cm^3 samples at 20, 40 and 60 hours during the lyophilisation process.

2.2.3. Tocopherol Quantification in Muscle Samples.

The α-tocopherol concentration in muscle samples was quantified by direct extraction as described by Rey, Daza, López-Carrasco & López-Bote (2006). Briefly, 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 analysed by reverse phase HPLC (HP 1100, equipped with a diode array detector; Agilent Technologies, Waldbronn, Germany) as described elsewhere (Rey & 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 analysed in duplicate. The α-tocopherol concentration in muscle was assessed on days 0 and 8 of refrigerated storage at 4°C.

2.2.4. TBARS Analysis of Muscle Samples.

The susceptibility of muscle homogenates to lipid oxidation induced by iron-ascorbate was determined by a modification of the method described by Kornburst & Mavis (1980). Muscle homogenates in 1.15% KCl (0.1 g/ml) were incubated at 37°C in 40 nM Tris-maleate buffer (pH 7.4) with 1 mM $FeSO_4$ and 2 mM ascorbic acid in a total volume of 10 ml. At fixed time intervals (0, 30, 60, 90 and 120 minutes), aliquots were removed for measurement of TBARS. Absorbance was measured at 532 nm. TBARS were expressed as mM malondialdehyde (MDA)/ g sample.

2.2.5. Glutathione Quantification

Reduced glutathione (GSH) and oxidised glutathione (GSSH) were quantified spectrophotometrically at 405 nm in deproteinised muscle using the corresponding diagnostic colourimetric kit (Arbor assays, USA). The concentration of oxidised glutathione was determined from the 2-vinylpyridine-treated samples read off 2-vinylpyridine-treated standard curve. Free glutathione (GSH) concentrations were obtained by subtracting the oxidised glutathione (GSSH) levels obtained from the 2-vinylpyridine-treated standard from non-treated standards and samples (total GSH). The concentrations obtained were expressed as μM of glutathione.

2.2.6. Instrumental Colour Analysis

Two-cm-thick samples were placed on trays and kept at 4ºC for colour measurement. Muscle colour was evaluated on days 1 and 7 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^*). Meat pigments (oxymyoglobin, deoxymyoglobin and metmyoglobin) were calculated by the isobestic wavelengths measured by means of the Chroma Meter. Hence, oxymyoglobin was calculated by the isobestic wavelengths of

deoxymyoglobin and metmyoglobin (610 nm/525nm); deoxymyoglobin by the isobestic wavelengths of oxymyoglobin and metmyoglobin (as the ratio of measurement at 474 nm/525 nm) and metmyoglobin by the isobestic wavelengths of deoxymyoglobin and oxymyoglobin (572 nm/ 525 nm) (Tang, Faustman, & Hoagland, 2004). Colour measurement and pigment concentrations in muscle were assessed on days 0, 4 and 8 of refrigerated storage at 4°C.

2.2.7. Separation of Myofibrillar Proteins by SDS-PAGE

Extraction of sarcoplasmic and myofibrillar proteins was performed as described by Molina and Toldrá (1992). Meat samples were homogenised with 30 mM phosphate buffer, pH 7.4 (dilution 1:10, w/v), using a masticator (IUL Instruments, Barcelona, Spain) for 4 min. After centrifugation at 10,000g for 20 min at 4°C, the supernatant containing the sarcoplasmic proteins was passed through glass wool and collected. The procedure was repeated twice in order to wash the pellet that was then resuspended in 9 vol of 100 mM phosphate buffer, pH 7.4, containing 0.7 M potassium iodide. The mixture was homogenised in the masticator for 8 min and then centrifuged at 10,000g for 20 min at 4°C. The supernatant was collected for analysis of myofibrillar proteins. The protein concentration of both supernatants containing sarcoplasmic and myofibrillar proteins was calculated by using bicinchoninic acid (Sigma Aldrich) as reagent and bovine serum albumin as standard.

Proteins were separated by 10% SDS-PAGE as described by Toldrá, Miralles and Flores (1992). The sarcoplasmic and myofibrillar extracts were mixed in a ratio of 1:1 with 50 mM Tris buffer, pH 6.8, containing 8 M urea, 2 M thiourea, 75 mM dithiothreitol, 3% (w/v) SDS and 0.05% bromophenol blue, heated at 100°C for 4 min and immediately chilled in ice and used for electrophoresis. The amount of protein injected into the gels was 12 µg per lane. Once proteins had been separated, gels were stained with coomassie brilliant blue R-250 (Laemmli, 1970). Standard proteins (Bio-Rad Laboratories, Inc., CA, USA) were run simultaneously for molecular mass identification. Stained bands in each gel were relatively quantified by densitometry, using the ImageJ Program (http://rsbweb.nih.gov/ij/).

2.2.8. Determination of Free Amino Acids

Free amino acids were determined following the procedure described by Aristoy and Toldrá (1991). Meat samples (*longissimus lumborum* muscle) were cut and minced, and 5 g was homogenised with 0.01 M HCl 1:5 (w/v) in a masticator (IUL Instruments) for 8 min at 4°C. After centrifugation at 10,000×g for 20 min at 4°C, the supernatant was filtered through glass wool and stored at −20°C until required. Once samples had thawed, 250 μL plus 50 μL of an internal standard solution (1 mM norleucine in 0.01 M HCl) was deproteinised using 2.5 volumes of acetonitrile and then centrifuged at 10,000×g for 5 min. Amino acid derivatisation was carried out with phenyl isothiocyanate (PITC) according to Bidlingmeyer, Cohen, Tarvin & Frost (1987). Supernatant (500 μL) was vacuum dried, mixed with 15 μL of methanol: 1 M sodium acetate: triethylamine (2:2:1) (v:v:v) and dried again. Then, 15 μL of methanol:water:triethylamine:PTIC (7:1:1:1, v:v:v:v) was added, held for 20 min and then dried. Derivatised amino acids were dissolved in 300 μL of 0.005 M phosphate buffer, pH 7.4, containing 5% acetonitrile, and analysed by reversed-phase HPLC in a 1200 Series Agilent chromatograph (Agilent, Palo Alto, CA, USA) using a Pico Tag® column (3.9×300 mm) (Waters Corporation, Milford, MA, USA). Separation was achieved in 55 min at 52°C using a gradient between two solvents: 70 mM sodium acetate at pH 6.55 containing 2.5% of acetonitrile (solvent A) and water:acetonitrile:methanol (40:45:15) (solvent B), as described by Armenteros, Aristoy, Barat and Toldrá (2012). Separated amino acids were detected at 254 nm.

2.2.9. Myofibrillar Fragmentation Index (MFI)

The myofibrillar fragmentation index was performed in duplicate as described by Culler et al. (1978). Forty ml cold MFI buffer (100 mM KCl, 20 mM potassium phosphate at pH 7, 1 mM $MgCl_2$ and 1 mM NaN_3 in distilled deionised water) was added to a blender containing the sample (4 g) and homogenised for 30 s. The homogenate was then centrifuged at 1000 x g for 15 min at 2°C and afterwards the supernatant was discarded. The pellet was resuspended in 40 ml cold MFI buffer and centrifuged at 1000 x g for 15 min. The pellet was again resuspended in 10 ml cold MFI buffer and vortexed until well mixed. Finally, the sample was poured through a polyethylene strainer to remove the connective tissue and the centrifuge tube was rinsed with an additional 10 ml cold MFI buffer. The protein content in each suspension was also measured by Biuret reaction. The extract (0.25 ml) was

mixed with 0.75 ml of MFI buffer and 4 ml of Biuret reagent (Sigma Aldrich, Alcobendas, Madrid). The mixture was vortexed and placed in the dark for 30 min. Bovine serum albumin (BSA) (Sigma Aldrich) was used as standard at concentrations of 0, 2.5, 5, 7.5 and 10 mg/ml. Once that the sample protein was determined, the amount of solution taken for MFI measurement contained approximately 0.5 mg protein / ml solution. MFI was measured spectrophotometrically using a Thermo Scientific Multiskan GO (Thermo Fisher, Alcobendas, Madrid) UV/VIS spectrophotometer at 540 nm, and it was expressed as absorbance of a myofibrillar protein solution (concentration 0.5 mg/ml) multiplied by 200.

2.2.10. Statistical Analysis

The experimental unit for analysis of all data was the yard. Data were analysed following a completely randomised design using the general linear model (GLM) procedure contained in SAS (version 9; SAS Inst. Inc., Cary, NC). A comparative analysis between means was conducted using the following orthogonal contrasts: (1) Control vs. others; (2) Vitamin E vs. others; (3) Se source effect; (4) Se dose effect (5) Source x dose interaction; (6) Control vs. Se; (7) Vitamin E vs. Se; (8) Control vs. vitamin E. Data were presented as the mean of each group and root mean square error (RMSE) together with significance levels (*P* value). Differences between means were considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Carcass Characteristics

Carcass characteristics are presented in Table 2. Carcass, *psoas major*, *longissimus thoracis et lumborum*, ham and shoulder weights were not statistically affected by dietary treatments. Total lean percentage was also unaffected; however, the percentage of dorsal fat was lower (P= 0.031) in those pigs supplemented with organic Se at high doses (SY-H) or vitamin E+Se. Kim et al. (2012) reported that Se (in the form of sodium selenite) inhibits adipocyte hypertrophy and abdominal fat accumulation in rats. Other authors found that a combination of Se and vitamin E supplements partially modulated fatty acid distribution (Douillet, Bost, Accominotti, Borson-Chazot & Ciavatti,

1998). In addition, Podszun et al. (2014) found that dietary α-tocopherol reduced high-fat-induced lipid accumulation in the liver. These effects were recently related to the potential increase in cell resistance to insulin (Pinto et al., 2012). The lack of effect observed in groups SS-L, SS-H and SY-L in the present study may have been due to an insufficient dose or administration time for observing these effects, and SY-H and vitamin E+Se were the most effective treatments to reduce fat percentage.

3.2 Electrical Conductivity, pH, Drip Loss and Meat Composition

The electrical conductivity (EC), pH and drip loss of muscle samples were unaffected by either selenium source or dietary vitamin E supplementation (Table 2 and 3). However, a marked effect was observed of organic selenium on total water losses and evolution during the lyophilisation process. Hence, meat samples from groups supplemented with the organic source of Se showed lower water losses after 20 hours (P=0.009), 40 hours (P=0.037) and 60 hours (P=0.045) when compared to the mineral source of Se. This effect resulted in lower water losses during the initial water loss process during lyophilisation (lower percentage at 20 h, P=0.027). It is interesting to note that the lowest water losses were observed in the SY-H group. Organic Se has been reported to increase water-holding capacity (Mahan et al., 1999; Zhan et al., 2007; Li et al., 2011; Lisiak et al., 2014), although other authors have not found any effect in pork (Castro-Rios & Narvaez-Solarte, 2013; Bobcek et al., 2004). However, these latter authors used doses of 0.3 mg/kg or lower. In contrast, Li et al. (2011) used three different doses of selenium-enriched yeast (0, 0.3, and 3.0 mg Se/kg) and found a clearly lower drip loss in meat samples from pigs supplemented with 3.0 mg/kg, whereas the use of 0.3 mg/kg resulted in intermediate drip loss values that were no different from those obtained for samples from non-supplemented pigs. Other authors who have observed an effect on drip loss used between 0.4-0.5 mg/kg in the form of organic Se (Lisiak et al., 2014) or a supplementation time of 40 days using doses of 0.3 mg/kg (Zhan et al., 2007). Mahan et al. (1999) fed pigs from 55 to 105 kg with organic selenium and found lower drip losses with a dose of 0.3 mg/kg when compared with the inorganic form. In the present research it was observed that differences were not statistically significant in drip loss of frozen muscle. The vitamin E+SS-supplemented group showed intermediate water loss values that were the same as in the other groups, and a lower percentage of water loss at 60 hours (P=0.0013)

when compared to the control group. According to the results of the present study, vitamin E plus a low dose of SS seems to have a more limited effect as regards reducing water loss in muscle samples when compared to organic Se at higher doses. Several authors have found that high levels of vitamin E reduce the amount of drip loss in pork (Ashgar et al., 1991; Cheah, Cheah & Krausgrill, 1995) in samples from pigs carrying the halothane gene (Cheah et al., 1995) or in pigs of unknown halothane status. These effects have been attributed to the antioxidant capacity of vitamin E, which protects membrane integrity and thus reduces water loss (Ashgar et al., 1991). Conversely, other studies have shown no effect or contradictory results (Dirinck, Winne, Casteels & Frigg, 1996; Jensen et al., 1997). Moreover, other authors have reported no beneficial effect on meat quality when using a combination of dietary vitamin E (60 mg/kg) and Se at low doses (0.2 mg) (Kawecka, Jacyno, Matysiak, Kolodziej-Skalska & Pietruszka, 2013).

3.3. Tocopherol Accumulation

The vitamin E concentration as affected by dietary treatment is presented in Table 3. As expected, muscle α-tocopherol content was affected by dietary α-tocopheryl acetate concentration, and groups supplemented with 100 mg/kg thus showed a higher concentration in meat samples (P=0.0001) than the other groups. Vitamin E was also affected by sample storage time, with all experimental groups showing a decrease. This is because vitamin E plays an important role in lipid antioxidant defence in the cell membrane (Buckley et al., 1995). Moreover, it is interesting to observe that control samples presented the lowest content of vitamin E, even though these had been supplemented with the same vitamin E dose as the Se-supplemented groups; however, differences between these groups were not statistically significant.

3.4. Lipid Oxidation and Antioxidant Status

To evaluate the oxidative stability of pork according to selenium source or dose when compared to a control or a vitamin E+SS-supplemented group, ferrous-induced oxidation was measured in muscle samples (Table 3). Muscle samples from pigs supplemented with dietary vitamin E at 100 mg/kg plus 0.2 mg SS/kg showed the lowest malondialdehyde (**MDA**) concentration at 0 min

(P=0.0004), 30 min (P=0.0001), 60 min (P=0.0010), 90 min (P=0.0001) and 120 min (P=0.0001) of incubation when compared to the other groups. Moreover, samples from pigs supplemented with vitamin E+SS had higher lipid stability (P< 0.05) than those supplemented with Se alone. Thus, MDA concentration in muscle was reduced by about 43% in vitamin E+SS-supplemented pigs and by 14% in animals fed a selenium-enriched diet when compared to control group. In addition, MDA production in the vitamin E+SS group was also reduced by 34% when compared to Se-supplemented pigs (P<0.005). α-Tocopherol which is mainly located in muscle membrane (Buckley et al., 1995) has been reported to be an effective antioxidant, capturing free radicals and other reactive substances (Dirinck, De Winne, Casyteels & Frigg, 1996). Selenium is also recognised as an essential trace element that plays an important role in antioxidant defences as a component of Se-dependent glutathione peroxidase (GSH-Px). This enzyme protects cells against damage caused by free radicals and lipoperoxides (Finkel & Holbrook, 2000). The effect of both compounds has also been studied and a synergistic action has been reported between vitamin E and Se to protect the cell (Saito, Yoshida, Akazawa, Takahashi & Niki, 2003). Harsini, Habibiyan, Moeini & Abdolmohammadi (2012) compared vitamin E (125 and 250 mg/kg) and Se (0.5 and 1 mg/kg) supplementation both separately and in combination (125 mg vitamin E/kg and 0.5 mg Se /kg), and found a higher reduction in MDA production in broilers fed with both compounds when compared to the control. However, they did not find differences between vitamin E or selenium when supplemented separately. Ebeid, Zeweil, Basyony, Dosoky & Badry (2013) also reported a similar antioxidant effect of organic selenium (0.3 mg /kg) and vitamin E (250 mg/kg) in rabbits, and the combination of both was the most effective treatment to reduce TBARS. In contrast, other authors have not found any positive effect of organic Se at low doses (0.3 mg/kg) on the oxidative stability of pork muscle (Krska et al., 2001).

In the present research, no differences were detected in TBARS production according to Se source or dose. In contrast, Zhan et al. (2006) reported a higher stability of muscle samples against lipid oxidation following supplementation with the organic form at a dose of 0.3 mg Se/kg administered for 40 days. Bobcek et al. (2004) also found an effect on TBARS production, but these authors used 0.3 mg of organic Se in pigs from 35 to 100 kg.

To confirm these results, antioxidant status was measured as the oxidised to reduced glutathione rate (GSSH/GSH). Glutathione reductase (GR) plays an important role in maintaining redox homeostasis by reducing GSSH (oxidised GSH) to GSH, and the increased GR activity produces more GSH under oxidative stress (Grant, Collisnson, Roe & Dawes, 1996). As observed before in TBARS content, meat from pigs supplemented with vitamin E+SS presented the lowest GSSH content (P=0.027) and the lowest GSSH/GSH ratio (P=0.054). Li et al. (2011) found a high correlation coefficient for TBARS content and glutathione peroxidase (GPX) activity. The results of the present study confirm that the vitamin E+SS group had the highest antioxidant capacity and that the different Se doses and sources had no effect on antioxidant capacity. Li et al. (2011) attributed the improvement in meat oxidative stability induced by Se to the protective effect of GPX against oxidative damage. In a proposed hierarchy of selenium usage, GSH-Px is one of the first needs to be satisfied (Hohe & Brigelius-Flohe, 2002). Mahan and Parret (1996) reported that although Se deposition in pig tissue was higher when the organic form of Se was fed, sodium selenite was more effective in attaining maximum GPX activity, particularly after feeding a diet with a low Se content over an extended period. Moreover, despite differences in GPX activity in organic selenium-supplemented animals, some studies have reported little or no potential effect of organic Se on improving the oxidative stability of meat (Juniper, Phipps, Ramos-Morales & Bertin, 2008; Juniper, Phipps & Bertin, 2011).

3.5. Colour and Pigment Stability of Muscle

Colour changes, measured as L* (lightness), a* (red colour), b* (yellow-green colour), chroma (colour intensity or saturation) and hue (tone) after 1, 4 and 8 days of refrigerated storage, are presented in Table 4. The main changes in colour were observed in the group supplemented with vitamin E+SS, which showed higher a* (P=0.032), b* (P=0.048) and colour intensity (P=0.037), whereas colour tone was lower (P=0.044) at day 4 of storage when compared to the other groups. Hence, the use of both compounds (vitamin E+SS) yielded meat samples with 25% higher redness and 8% more colour intensity than the use of organic or inorganic Se at high or low dose without vitamin E supplementation. The positive effects of dietary vitamin E have previously been reported by others

(Faustman et al., 1996; Monahan, Buckley, Morrissey, Lynch & Gray, 1992). Monahan et al. (1992) found higher redness in pork chops from animals fed a vitamin E-enriched diet when compared to a control. Furthermore, the combination of vitamin E and Se has also yielded colour improvements (Krska et al., 2001). However, neither Se source nor dose were observed to exert an effect in the present study, which is consistent with the results found for the TBARS numbers and may possibly be explained by the short supplementation period. Other authors have not found any effect of selenium on meat colour (Lisiak et al., 2014). Juniper et al. (2011) have suggested that once the Se content of tissue exceeds the requirements of antioxidant enzymes, further increases in tissue Se do not result in any noticeable improvement in meat quality. However, others found a favourable effect of the organic form on colour parameters when compared with the inorganic form (Zhan et al., 2007; Bobcek et al., 2004; Mahan et al., 1999).

The concentration of myoglobin was also quantified because it is the main heme protein responsible for meat colour (Table 5). As explained before, the main effect observed on meat pigments was found when pigs were supplemented with vitamin E at 100 mg/kg and low doses of inorganic Se. Thus, pigs from the vitamin E+SS group presented higher DeoxyMb (P=0.04) and OxyMb (0.04) at day 4 and a lower MetMb to DeoxyMb ratio (P=0.04) when compared with the other groups. The vitamin E+SS group also showed higher DeoxyMb (P=0.02) and OxyMb (P=0.025) at day 4 and DeoxyMb at day 8 (P=0.05) when compared with those groups supplemented with Se. These results are consistent with those observed for colour parameters and TBARS numbers. Furthermore, a Se source effect was observed whereby pigs fed SY had a lower MetMb to DeoxyMb ratio (P=0.03) than those supplemented with SS.

3.6. Myofibrillar Proteins and Free Amino Acids

The myofibrillar protein pattern is presented in Figure 1. Electrophoresis profiles were affected by the dietary treatment and the storage time. Meat storage essentially affected the troponin T, tropomyosin and 150 kDa bands, which partially disappeared after 7 days of storage. Other authors found similar results for storage time (Martinaud et al., 1997; Santé-Lhoutellier, Engel, Aubry & Gatellier, 2008) in bovine and lamb muscle. Martinaud et al. (1997) also found a concomitant

appearance of a 30 kDa band which was considered as a proteolysis index. However, as with Santé-Lhoutellier et al. (2008), we did not detect this compound.

The main effect observed between treatments was the greater decrease in MLC2, Tn-I, tropomyosin and Tn-T bands in control and SS-L treatments after 7 days of refrigerated storage when compared to the others. In addition, the 150 kDa band disappeared to a lesser extend in SS-H, SY-L, SY-H and VITE+SS after 7 days of storage. The disappearance of the myosin, troponin and tropomyosin bands could correspond to oxidation processes. Hence, dietary enrichment with organic selenium, synthetic selenium at high doses and vitamin E+SS exerted a strong effect on myofibrillar proteins in the present study. On the other hand, bands 98 and 105, which were already observed at day 2 of storage, were more marked after day 7 of storage in treatments SY-L, SY-H and VITE-SS. The effect of dietary vitamin E supplementation on myosin and tropomyosin bands has previously been reported (Aksu, Aktas, Kaya & Macit, 2005); however, no studies have investigated the effect of selenium.

Also noteworthy was the presence of a higher number of bands between tropomyosin and Tn-I bands in the VITE+SS group, which may be the result of tropomyosin fragmentation or may be an indicator of lower losses or myofibrillar oxidation, bearing in mind the high antioxidant power of this diet. Aksu, Aktas, Kaya & Macit (2003) also found a similar effect of vitamin E supplementation on myofibrillar proteins when using 45 mg/day for 75 days in lambs. Servais, Letexier, Favier, Duchamp & Desplanches (2007) reported that vitamin E supplementation decreases the rate of muscle proteolysis by reducing the expression of calpains, caspases-3, -9 and -12, and E3 ubiquitin ligases. Calpains are non-lysosomal Ca2+-activated cysteine proteases that cleave titin and nebulin at sites near the Z-disc (Goll, Thompson, Li, Wei & Cong, 2003). Thus, the calpain system plays an important role in regulating proteolysis of muscle proteins under post-mortem conditions (Lonergan, Huff-Lonergan, Wiegand & Kriese-Anderson, 2001). Additional new bands between MLC-1 and Tn-C and between MLC2 and MLC3 appeared in the SeY-H group after 7 days of storage, but not in the other groups. These new compounds may arise from the decomposition of myosin and troponin chains and may be indicators of a higher proteolysis activity in this group.

The free amino acid content of pork samples is shown in Tables 7 and 8. As expected, free amino acid content increased with meat ageing and was due to the action of muscle aminopeptidases (Moya et al., 2001). The group supplemented with vitamin E+SS had the lowest content of serine (SER) (P=0.011) and anserine (ANS) (P=0.006) at day 2 of storage and of SER (P=0.035), ANS (P=0.018) and carnosine (CAR) (P=0.009) at day 7 when compared to the other groups. In contrast, it is also of interest to observe that essential amino acids were higher in the group supplemented with VITE+SS when compared with the others. Hence, PRO (P=0.0001), TRP (P=0.0063), and PHE (P=0.0062) at day 2 and day 7 (P= 0.0001; P=0.0001 and P=0.0004, respectively) were higher in meat from pigs supplemented with the VITE+SS-enriched diet when compared with the other groups. These free amino acid contents (PRO, PHE, and TRP) were also lower in Se-enriched groups when compared with the group supplemented with VITE+SS, and no effects of the Se source or dose were found, which would indicate that the increase in the concentration of these essential amino acids was mainly due to vitamin E supplementation. This phenomenon has not been observed before. Taking into account the higher antioxidant power of the VITE+SS supplement found in the present study for MDA production, colour changes and myofibrillar protein, this result might be explained by a higher protective effect of vitamin E against protein and amino acid oxidation (Mercier, Gatellier & Renerre, 2004). This protective effect of vitamin E would also maintain calpain activation post-mortem, which would increase proteolysis of filament proteins in meat.

The Se source also affected the free amino acid content of meat. Meat from pigs supplemented with organic Se had higher content of glycine (GLY) and carnosine (CAR) at day 2 and also tended to have higher taurine (TAU) (P=0.098) and anserine (ANS) (P=0.051) at day 2 and alanine (ALA) (P=0.078) at day 7 when compared with the inorganic form. The dose effect was only observed in ALA content, which increased with the Se supplementation dose (P=0.036). Moreover, dietary Se supplementation resulted in higher SER (P=0.027 and P=0.026 at days 2 and 7, respectively), ANS (P=0.0005 and P=0.012 at days 2 and 7, respectively) and CAR (P=0.016 at day 2) when compared to vitamin E supplementation. As indicated before, no previous information exists on the effect of Se source or dose on muscle proteolysis. Carnosine is an important natural water-soluble muscle dipeptide that can contribute to the inactivation of lipid oxidation catalysts and free radicals in the

sarcoplasm (Decker & Crum, 1993). The different proteolytic effect of the Se sources should be highlighted since a higher proteolysis may be partially responsible for the increased water-holding capacity (WHC) observed in groups supplemented with organic Se when compared to the inorganic Se. Hence, Kristensen and Purslow (2001) hypothesised that the increase in meat WHC during storage time was due to post-mortem degradation of cytoskeletal proteins. They further postulated that the elution water flow rate is inversely related to the quantity of proteolysed cytoskeletal proteins with time post-mortem. In turn, Lonergan & Lonergan (2005) reported that the degradation of proteins such as desmin, talin or vinculin in the early post-mortem period allows water that is expelled from intramyofibrillar spaces to remain in the cell for a longer period of time. Consequently, the results of the present study would support the hypothesis that early post-mortem proteolysis of intermediate filament proteins can minimise the flow of water from within the cell to the drip channels. However, other studies showed that not always protein degradation could increase water holding capacity of pork (Lawson, 2004; Zhang et al., 2006).

However, given that these proteins are substrates of calpains, which are directly involved in regulating proteolysis, and that it has been reported that calpain expression is reduced by vitamin E supplementation (Servais et al., 2007), higher drip loss would be expected in pigs that received a vitamin E-enriched diet. Conversely, it has also been reported that protein oxidation may reduce the functionality of calpains; thus, vitamin E might also protect muscle enzymes. This possible double effect described for vitamin E in inhibiting calpain oxidation and expression could be responsible for the intermediate effects observed for water loss in the present study. The possible effect of higher free-essential amino acid content is unknown. These differences in muscle proteolysis by dietary vitamin E enrichment might in part explain the controversial effects of dietary vitamin E supplementation on drip loss reported in many studies in the literature. However, numerous other factors that could affect the calpain system must be taken into consideration, and further research is required to confirm these effects.

3.7. *Myofibril Fragmentation Index (MFI)*

In order to confirm the effect of Se on meat proteolysis, the myofibrillar fragmentation index (MFI) was measured (Table 8). At day 2 of storage, MFI was not modified by the dietary treatment. At day 8, the MFI was neither statistically affected. However, SeY and VITE+SS groups had the highest MFI values at day 8, even though differences were not statistically different (P=0.147 and P=0.125, respectively). The increase in MFI values with meat ageing has been reported by others (Dosler, Polak, Zlender, Gasperlin, 2007), since there is a relationship between muscle proteolysis and MFI values (Kristensen et al., 2002; Koohmaraie, Whipple, Kretchmar, Crouse & Mersmann, 1991). These results would be in accordance with the myofibrillar protein oxidation and free amino acid content described above. Hence, meat from groups supplemented with SeY and VITE+SS presented higher muscle proteolysis, which would explain the lower or intermediate water losses observed for these groups.

In conclusion, dietary selenium source at 0.2 or 0.4 mg/kg administered for 26 days did not modify MDA production, GSSH concentration or colour parameters of meat. However, organic Se effectively increased WHC and post-mortem muscle proteolytic activity. The administration of 0.2 mg/kg SS plus 100 mg VITE/kg was the most efficient treatment for stabilising colour, reducing MDA and GSSH production and decreasing myofibrillar protein hydrolysis/oxidation; however, this dietary supplementation was not as effective as organic Se administration in improving WHC. This result would confirm the relation between muscle proteolysis and WHC.

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5. REFERENCES

Aksu, M. I., Aktas, N., Kaya, M., & Macit, M. (2003). The effects of vitamin E supplementation on the myofibrillar protein of male Morkaraman lambs. *Turkish Journal of Veterinary and Animal Sciences*, 27, 987-990.

Aksu, M. I., Aktas, N., Kaya, M., & Macit, M. (2005). Effects of vitamin E supplementation on myofibrillar proteins of fat-tailed awassi male lambs. *Journal of Muscle Foods*, 16, 177-183.

Andersen, H. J., Oksbjerg, N., Young, J.F., & Therkildsen, M. (2005). Feeding and meat quality- a future approach. *Meat Science*, 70, 543-554.

Aristoy, M.C., & Toldrá, F. (1991). Deproteinization techniques for HPLC amino acids analysis in fresh pork muscle and dry-cured ham. *Journal of Agricultural and Food Chemistry*, 39, 1792-1795.

Aristoy, M. C., & Toldrá, F. (1995). Isolation of flavor peptides from raw pork meat and dry-cured ham. In G. Charalambous, *Food flavors:generation analysis and process influence* (pp. 1323-1344). Amsterdam, The Netherlands: Elsevier Science, BV.

Armenteros, M., Aristoy, M. C., Barat, J. M., & Toldrá, F. (2012). Biochemical and sensory changes in dry-cured ham salted with partial replacements of NaCl by other chloride salts. *Meat Science*, 90, 361-367.

Ashgar, A., Gray, J. I., Miller, E. R., Ku, P. K., Booren, A. M., & Buckley, D. J. (1991). Influence of supranutritional vitamin E supplementation in the feed on swine growth, performance and deposition in different tissues. *Journal of the Science of Food and Agriculture*, 57, 31-41.

Bidlingmeyer, B. A., Cohen, S. A., Tarvin, T. L., & Frost, B. A. (1987). New rapid high sensitivity analysis of amino acids in food type samples. *Journal of Association of Analytical Chemistry*, 70, 241–247.

Bobcek, B., Lahucky, R., Mrazova, J., Bobcek, R., Novotna, K., & Vasicek, D. (2004). Effects of dietary organic selenium supplementation on selenium content, antioxidative status of muscles and meat quality of pigs. *Czech Journal of Animal Science*, 49, 411-417.

BOE (2013). RD 53/2013, de 21 de octubre por la que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. Spain. *Boletín Oficial del Estado 252*, 34367-34391.

Boles, J. O., Cisneros, R. J., Weir, M. S., Odom, J. D., Villafranca, J. E. & Dunlap, R. B. (1991). Purification and characterization of selenomethionyl thymidylate synthase from *Escherichia coli*; comparison with the wild-type enzyme. *Biochemistry* 30: 11073–11080.

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V., & Geraert, P. A. (2013). Comparative study of a new organic selenium source vs. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. *British Journal of Nutrition, 110,* 617-624.

Buckley, D. J., Morrissey, P. A. & Gray, J. I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science,* 73, 3122-313.

Carling, D. (2004). The AMP-activated protein kinasecascade– a unifying system for energy control. *Trends Biochemical Science,* 29, 18–24.

Castro-Ríos, K., & Narvaéz-Solarte, W. (2013). Sensory quality and cooking loss in pork meat: effect of sex and source of selenium. *Biotecnología en el sector agropecuario y agroindustrial, 11*(1), 130-135.

Cheah, K., S., Cheah, A. M., & Krausgrill, D. I. (1995). Effect of dietary supplementation of vitamin E on pig meat quality. *Meat Science*, 39, 255-264.

CIE (1976). Recommendations on uniform colour spaces-colour difference equations-psychometric colour terms. *Commission Internationale de l'Eclairage, Suppl. 2 to CIE*. Publication nº 15 (E-1.31) 197/ (TC-1.3). Bureau de la CIE, Paris.

Ciobanu D., Bastiaansen J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophosphate-activated g3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, 159, 1151–1162.

Culler, R. D., F. C. Parrish, F.C. Jr., Smith, G. C., & Cross, H. R. (1978). Relationship of myofibril fragmentation index to certain chemical, physical, and sensory characteristics of bovine longissimus muscle. *Journal of Food Science,* 43, 1177.

Decker, E. A., & Crum, A.D. (1993). Antioxidant activity of carnosine in cooked ground pork. *Meat Scienc*e, 34, 245-253.

Dirinck P, De Winne A, Casyteels M, & Frigg M. (1996). Studies on vitamin E and meat quality. 1. Effect of feeding high vitamin E levels on time related pork quality. *Journal of Agriculture and Food Chemistry*, 44, 65-68.

Dosler, D., Polak, T., Zlender, B., Gasperlin, L. & (2007). Relation of myofibril fragmentation to textural and chemical parameters of aged pork Longissimus dorsi. *Acta agriculturae Slovenica*, 90 (1), 5-16

Douillet, C., Bost, M., Accominotti, M., Borson-Chazot, F., & Ciavatti, M. (1998). Effect of selenium and vitamin E supplementation on lipid abnormalities in plasma, aorta, and adipose tissue of zucker rats. *Biological Trace Element Research*, 1998, 221-236.

Ebeid, T. A., Zeweil, H. S., Basyony, M. M., Dosoky, W. M., & Badry, H. (2013). Fortification of rabbit diets with vitamin E or selenium affects growth performance, lipid peroxidation, oxidative status and immune response in growing rabbits. *Livestock Science*, 155, 323-331.

Enfält A. C., von Seth G., Josell Å, Lindahl G., Hedebro-Velander I., Braunschweig M., Andersson L., & Lundstörm K. (2006). Effects of a second mutant allele (V199I) at PRKAG3 (RN) Locus on carcass composition in pigs. *Livestock Science*, 99, 131–139.

Faustman, C., & Wang, F. (2000). Potential mechanisms by which vitamin E improves oxidative stabililty of myoglobin. In E.A. Decker, C. Faustman, & C. J. López-Bote (Eds.) Antioxidants in muscle foods-nutritional strategies to improve quality (pp. 135-152). Toronto: John Wiley & Sons, Inc.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*, 239-247.

Flores, M., Aristoy, M. C., & Todrá, F. (1996). HPLC purification and characterization of soluble alanyl aminopeptidase from porcine skeletal muscle. *Journal of Agricultural and Food Chemistry*, 44, 2578-2583.

Goll, D.E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiological Review*, 83, 731-801.

Grant, C. M., Collisnson, L. P., Roe, J. H., & Dawes, I. W. (1996). Yeast glutathione reductase is required for protection against oxidative stress and is a target gene for yap-1 trascrptional regulation. *Molecular Microbiology*, 21, 171-179.

Harsini, S. G., Habibiyan, M., Moeini, M. M., & Abdolmohammadi, A. R. (2012). Effects of dietary selenium, vitamin E, and their combination on growth, serum metabolites, and antioxidant defense system in skeletal muscle of broilers under heat stress. *Biological Trace Element Research*, 148, 322-330.

Honikel, K. O. (1997). Reference methods supported by the OECD and their use in Mediterranean meat products. *Food Chemistry, 59*, 573-582.

Jang, Y. D., Choi, H. B., Durosoy, S., Schlegel, P., Choie, B. R., & Kim, Y. Y. (2010). Comparison of bioavailability of organic selenium sources in finishing pigs. *Asian Australasian Journal of Animal Sciences, 23*, 931-936.

Jensen, C., Guidera, J., Skowgaard, I. M., Staun, H., Skibsted, L. H., Jensen, S. K., Moller, A. J., Buckley, J., & Bertelsen, G. (1997). Effects of dietary alpha-tocopheryl acetate supplementation on alpha-tocopherol deposition in porcine m. psoas major and m. longissimus dorsi and on drip loss, color stability and oxidative stability of pork meat. *Meat Science*, 45, 491-500.

Juniper, D. T., Phipps, R. H., Ramos-Morales, E. & Bertin, G. (2008). Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in beef cattle. *Journal of Animal Science, 86*, 3100-3109.

Juniper, D. T., Phipps, R. H., & Bertin, G. (2011). Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in commertial-line turkeys. *Animal, 5*, 1751-1760.

Kawecka, M., Jacyno, E., Matysiak, B., Kolodziej-Skalska, A., & Pietruszka, A. (2013). Effects of selenium and vitamin E supplementation on selenium distributions and meat quality of pigs. *Acta Agriculturae Scandinavica Section A-Animal Science, 63*, 194-200.

Kim, J. E., Choi, S. I, Lee, H. R., Hwang, I. S., Lee, Y. L., An, B. S., Lee, S. H., Kim, H. J., Kang, B. C., & Hwang, D. Y. (2012). Selenium significantly inhibits adipocyte hypertrophy and abdominal

fat accumulation in OLETF rats via induction of fatty acid β-oxidation. *Biological Trace Element Research*, 150, 360-370.

Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D., & Mersmann, H. J. (1991). Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, 69, 617-624.

Kristensen, L., Therkildsen, M., Riis, B., Sorensen, M. T., Oksbjerg, N., Purslow, P. P. & Ertbjerg, P. (2002). Dietary-induced changes of muscle growth rate in pigs: Effects on in vivo and post-mortem muscle proteolysis and meat quality. *Journal of Animal Science*, 80, 2862-2871.

Kristensen, L., & Purslow, P. P. (2000). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. *Meat Science*, 58, 17-23.

Kronbrust, D. J., & Mavis, R. D. (1980). Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. *Lipids*, 15, 315-322.

Krska, P., Lahucky, R., Kuchenmeister, U., Nurnberg, K., Palanska, O., Bahelka, I., Kuhn, G., & Ender, K. (2001). Effects of dietary organic selenium and vitamin E supplementation on post mortem oxidative deterioration in muscles of pigs. *Archives of Animal Breeding*, 44: 193-201.

Laemmly, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.

Lametsch, R., Roepstorff, P., Moller, H.S., & Bendixen, E. (2004). Identification of myofibrillar substrates for μ-calpain. *Meat Science*, 68, 515-521.

Lawson, M.A. (2004). The role of integrin degradation in post-mortem drip loss in pork. *Meat Science*, 68, 559-566

Li, J., Zhou, J., Zhao, H., Lei, X., Xia, Z., Gao, G., & Wang, K. (2011). Enhanced water-holding capacity of meat was associated with increased Sepw1 gene expression in pigs fed selenium-enriched yeast. *Meat Science, 87*, 95-100.

Lindahl G., Enfält A. C., Von Seth, G., Josell, Å, Hedebro-Velander, I., Andersen, H. J., Andersson L., Lundström, K. (2004). A second mutant allele (V199I) at the PRKAG3 (RN) locus. – I. Effect on technological meat quality of pork loin. *Meat Science*, 66, 609-619. Milan

Lisiak, D., Janiszewski, P., Blicharski, T., Borzuta, K., Grzeslowiak, E., Lisiak, B., Powalowski, K., Samardakiewicz, L., & Batorska, M. (2014). Effect of selenium supplementation in pig feed on slaughter value and physicochemical and sensory characteristics of meat. *Annals of Animal Science, 14*, 213-222.

Lonergan, S. M., Huff-Lonergan, E., Wiegand, B. R., & Kriese-Anderson, L. A. (2001). Postmortem proteolysis and tenderization of top loin steaks from brangus cattle. *Journal of Muscle Foods*, 12, 121-136.

Lonergan, E. & Lonergan, S. T. (2005). Mechanisms of water-holding capacity of meat: The role of post-mortem biochemical and structural changes. *Meat Science*, 71, 194-204.

Mahan, D.C., & Parret, N.A. (1996). Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine. *Journal of Animal Science*, 74, 2967-2974.

Mahan, D. C., Cline, T. R., & Richert, B. (1999). Effects of dietary levels of selenium-enriched yeast and sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum glutathione peroxidase activity, carcass characteristics, and loin quality. *Journal of Animal Science, 77*, 2172-2179.

Mahan, D. C., Azain, M., Crenshaw, T. D., Cromwell, G. L., Dove, C. R., Kim, S. W., Lindemann, M. D., Miller, P. S., Petigrew, J. E., Stein, H. H., & Wan Heugten, E. (2014). Supplementation of organic and inorganic selenium to diets using grains grown in various regions of the United States with differing natural Se concentrations and fed to grower-finisher swine. *Journal of Animal Science, 92*, 4991-4997.

Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry*, 45, 2481-2487.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S. & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science*, 82, 1195-1205.

Mercier, Y., Gatellier, P., & Renerre, M. (2004). Lipid and protein oxidation in vitro, and antioxidant potential in meat form Charolais cows finished on pasture or mixed diet. *Meat Science*, 66, 467-473.

Mikulski, D., Jankonwski, J., Zdunczyk, Z., Wroblewska, M., Sartowska, K., & Majewska, T. (2009). The effect of selenium source on performance, carcass traits, oxidative status of the organism and meat quality of turkeys. *Journal of Animal and Feed Sciences, 18*, 518-530.

Mitchelhill K. I., Stapleton D., Gao G., House C., Michell B., Katsis F., Witters L. A., & Kemp B. E. (1994). Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. Journal of Biological Chemistry, 269, 2361-2364.

Molina, I., & Toldrá, F. (1992). Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *Journal of Food Science*, 57, 1308–1310.

Monahan, F. J., Buckley, D. J., Morrissey, P. A., Lynch, P. B., & Gray, J. I. (1992). Influence of dietary fat and α-tocopherol supplementation on lipid oxidation in pork. *Meat Science*, 31, 229-241.

Moya, V. J., Flores, M., Aristoy, M. C., & Toldrá, F. (2000). Pork meat quality affects peptide and amino acid profiles during the ageing process. *Meat Science*, 58,197-206.

National Research Council, NRC (2012). Nutrient Requirement of Swine, eleventh ed. NRC, National Academy Press, Washington DC, USA.

Otto G., Roehe R., Looft H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S. & Kalm, E. (2006). Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. *Meat Science*, 75,185-195.

Payne, R. L., & Southern, L. L. (2005). Comparison of inorganic and organic selenium sources for broilers. *Poultry Science, 84*, 898-902.

Pinto, A., Juniper, D. T., Sanil, M., Morgan, L., Clark, L., Sies, H., & Steinbrenner, H. (2012). Supranutritional selenium induces alterations in molecular targets related to energy metabolism in skeletal muscle and visceral adipose tissue of pigs. *Journal of inorganic biochemistry, 114*, 47-54.

Podszun, M., Grebenstein, N., Spruss, A., Schlueter, T., Kremoser, C., Bergheim, I., & Frank, J. (2014). Dietary α-tocopherol and atorvastatin reduce high-fat-induced lipid accumulation and down-regulate CD36 protein in the liver of guinea pigs. *Journal of Nutritional Biochemistry*, 25, 573-579.

Rey, A. I., Daza, A., López-Carrasco, C., & López-Bote, C. J. (2006). Quantitative study of the alpha- and gamma-tocopherols accumulation in muscle and backfat from Iberian pigs kept free-range as affected by time of free-range feeding or weight gain. *Animal Science, 82,* 901-908.

Rey, A .I., & López-Bote, C. J. (2014). Alpha-tocopherol stereoisomer analysis as discriminant method for distinguishing Iberian pig feed intake during the fattening phase. *Food Chemistry, 142,* 342-348.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of calpain. *Journal of Animal Science*, 82, 3254-3266.

Saito, Y., Yoshida, Y., Akazawa, T., Takahashi, K., & Niki, E. (2003). Cell death caused by selenium deficiency and protective effect of antioxidants. *Journal of Biological Chemistry, 278,* 39428-39434.

Santé-Lhoutellier, V., Engel, E., Aubry, L., and Gatellier, P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Science, 79:* 777-783.

SAS. (1999). SAS user's guide statistics. Statistical Analysis Systems Institute Inc, Cary, NC.

Schrauzer, G. N. (2000). Selenomethionine: a review of its nutricional significance, metabolism and toxicity. *The Journal of Nutrition, 130,* 1653-1656

Servais, S., Letexier, D., Favier, R., Duchamp, D., & Desplanches, D. (2007). Prevention of unloading-induced atrophy by vitamin E supplementation. Links between oxidative stress and soleus muscle proteolysis?. *Free Radical Biology andMedicine,* 42, 627-635.

Shen H. M., Yang, C. F., Liu, J., & Ong, C. N. (2000). Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells. Free Radical Biology and Medicine, 28, 1115-1124

Spanier, A. M., McMillin, K. W., & Miller, J. A. (1990). Enzyme activity levels in beef: effect of post-mortem aging and end-point temperature. *Journal of Food Science*, 55, 318-322.

Surai, P. F. (2006). Selenium absorption and metabolism. In *Selenium nutrition and health*, (pp. 161-171). Nottingham University Press, Nottingham, UK.

Suzuki, K. T., & Ogra, Y. (2002). Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. *Food Additives and Contaminants, 19* (10), 974-983.

Tang, J., Faustman, C., & Hoagland, T. A. (2004). Krzywicki Revisited: Equations for Spectrophotometric Determination of Myoglobin Redox Forms in Aqueous Meat Extracts. *Journal of Food Science*, 69, 717-720

Toldrá, F., Miralles, M. C., Flores, J. (1992). Protein extractability in dry-cured ham. *Food Chemistry*, 44, 391–399.

Zhan, X., Wang, M., Zhao, R., Li, W., & Zu, Z. (2007). Effects of different selenium source on selenium distribution, loin quality and antioxidant status in finishing pigs. *Animal Feed Science and Technology, 132*, 202-211.

Zhang, W. G., Lonergan, S. M., Gardner, M. A., & Huff-Lonergan, E. (2006) Contribution of post-mortem changes of integrin, desmin and u-calpain to variation in water holding capacity of pork. *Meat Science*, 74, 578-585.

Figure caption 1.- Myofibrilar proteins of muscle samples as affected by dietary treatments (1: CONTROL, 2: SS-L; 3: SS-H; 4: SY-L; 5: SY-H; 6: VITE-SS).

Figure 1.-

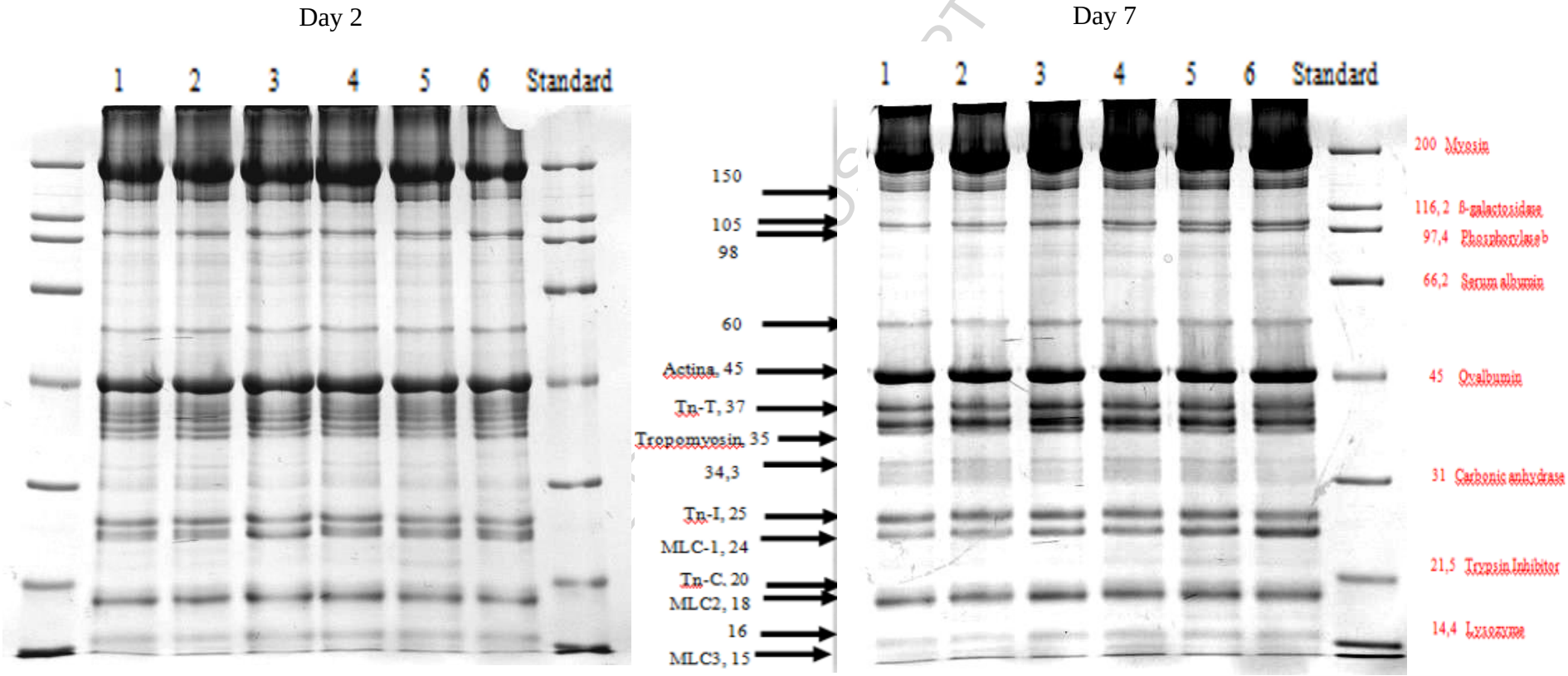

Table 1.- Ingredients, major nutrients and analysed composition of the experimental diets

	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE
Ingredients[1]						
α-Tocopherol, mg/kg	15	15	15	15	15	100
Selenium (sodium selenite), mg/kg	0	0,2	0,4	0	0	0,2
Selenium (Selplex), mg/kg	0	0	0	0,2	0,4	0
Major nutrients						
Dry matter, %	89,8	89,8	89,8	89,8	89,8	89,8
Crude protein, %	13,2	13,2	13,2	13,2	13,2	13,2
Fat, %	6,2	6,2	6,2	6,2	6,2	6,2
Crude fiber, %	4,2	4,2	4,2	4,2	4,2	4,2
Ash, %	4,4	4,4	4,4	4,4	4,4	4,4
Starch, %	46,3	46,3	46,3	46,3	46,3	46,3
Lysine dig, %	0,7	0,7	0,7	0,7	0,7	0,7
Met dig, %	0,2	0,2	0,2	0,2	0,2	0,2
Met + Cis dig, %	0,4	0,4	0,4	0,4	0,4	0,4
Thr dig, %	0,4	0,4	0,4	0,4	0,4	0,4
EN, kcal/kg	2449	2449	2449	2449	2449	2449
Analysed composition						
α-Tocopherol, µg/g	47,62	41,53	51,74	40,95	42,81	175,09
Selenium, mg/kg	0,0004	0,19	0,42	0,22	0,39	0,21

[1] Ingredients (g/kg): 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; Premix *[,] 0.20; L-Threonine, 0.08; Bactericide, 0.05; Fungicide, 0.03.

[*] Premix (per Tn of finished diet): Vitamin A: 8000000 IU; Vitamin D_3: 1800000 IU; **Vitamin E** (all rac α-tocoferyl-acetate)**: 15000 mg; Vitamin B_1: 0.5 g; Vitamin B_2: 1.5 g; Vitamin B_{12}: 12 mg; Vitamin B_6: 1 g; Nicotinic acid: 15 g; Calcium Pantothenate: 10 g; Vitamin K_3: 0.5 g; Choline chloride: 100 g; Fe (ferrous carbonate): 70 g; Cu (pentahidrated sulphate): 15 g; Co (hidrate carbonate): 0.05 g; Zn (oxide): 100 g; Mn (oxide): 40 g; I (potasium iodure): 0.5 g; **Se (sodium selenite)***: 0.2 g (SS-L) or 0.4 g (SS-H)**; 6-fitase EC 4-12: 500000 FTU; xilanase 4-8: 24000000 BXU; BHT E321: 2 g; citric acid E330: 6,9 g; sodium citrate E331: 0.2 g; sepiolite: 150 g.

Table 2.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on electric conductivity (EC) and pH after slaughter in muscle samples from pigs fed the experimental diets.

	CONTROL		SS-L		SS-H		SY-L		SY-H		VITE+SS		RMSE[1]	P[2]
Carcass characteristics														
Carcass weight, kg	95,23		94,48		94,37		96,01		91,85		91,74		5,942	0,2761
Psoas major weight, kg	0,59		0,58		0,59		0,59		0,60		0,58		0,035	0,5435
Longissimus thoracis et lumborum weight, kg	4,18	ba	4,11	ba	4,26	a	4,21	ba	4,14	ba	4,03	b	0,249	0,1941
Ham weight, kg	12,64		12,55		12,56		12,99		12,33		12,43		0,881	0,3362
Shoulder weight, kg	8,06	ba	7,93	ba	8,01	ba	8,07	a	7,95	ba	7,86	b	0,252	0,1984
Total lean meat, %	57,00		56,24		56,46		56,59		57,86		57,80		2,728	0,4502
Dorsal fat, %	24,81	ba	26,12	a	25,00	ba	24,93	ba	22,47	b	22,91	b	3,323	**0,0310**
Meat characteristics														
pH at 3 h	6,40		6,37		6,39		6,48		6,45		6,44		0,253	0,8561
pH at 20 h	5,69		5,75		5,67		5,68		5,66		5,67		0,158	0,6404
pH at 36 h	5,71		5,76		5,68		5,68		5,68		5,69		0,154	0,6436
EC at 20 h	2,47		2,75		2,41		2,22		2,30		2,11		1,214	0,7884
EC at 36 h	3,83		4,19		3,78		3,47		4,47		3,59		1,412	0,4053

[1] RMSE: Root of the mean square error

[2] P: Differences were statistically significant when P<0.05

Table 3.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on moisture, drip loss and water loss during lyophilisation in muscle samples from pigs fed the experimental diets.

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
Moisture (%)															
Day 0	73,90	73,76	73,58	73,48	73,45	73,04	1,37	NS[4]	NS	NS	NS	NS	NS	NS	NS
Day 7	73,63	73,37	73,22	72,95	72,42	73,35	1,16	NS	NS	NS	NS	NS	NS	NS	NS
Drip loss (g drip loss / 100 g sample)															
Fresh	6,83	7,47	6,74	6,61	6,67	7,46	1,53	NS	NS	NS	NS	NS	NS	NS	NS
Frozen	16,05	16,11	14,77	15,03	13,98	14,76	2,41	NS	NS	NS	NS	NS	NS	NS	NS
Water loss during lyophilization (g water/ 100 sample)															
20 h	71,87	71,65	70,62	68,98	68,15	69,28	2,87	**0,0470**	NS	**0,0090**	NS	NS	**0,0600**	NS	NS
40 h	72,84	73,18	72,35	72,09	71,81	72,41	1,15	NS	NS	**0,0371**	NS	NS	NS	NS	NS
80 h	73,34	73,55	72,78	72,46	72,26	72,75	1,18	NS	NS	**0,0450**	NS	NS	NS	NS	NS
pc_20h[3]	97,99	97,40	97,03	95,20	94,27	95,19	3,26	**0,0700**	NS	**0,0270**	NS	NS	NS	NS	**0,0750**
pc_40h	1,33	2,09	2,39	4,29	5,11	4,32	3,32	**0,0630**	NS	**0,0310**	NS	NS	**0,0909**	NS	**0,0619**
pc_60h	0,68	0,51	0,58	0,51	0,62	0,48	0,14	**0,0100**	**0,0538**	NS	**0,0550**	NS	**0,0244**	NS	**0,0013**

[1] RMSE: Root of the mean square error

[2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when $P < 0.05$

[3] Percentage of drip losses at 20 h; [4] NS: Not statistically significant

Table 4.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on α-tocopherol (µg/g), ferrous-induced oxidation (mg MDA/g meat) and muscle glutathione (µM/g) in muscle samples from pigs fed the experimental diets.

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
Vitamin E (µg/g)															
day 0	1,88	2,11	2,12	2,00	1,97	3,28	0,54	**0,0384**	**0,0001**	NS	NS	NS	NS	**0,0001**	**0,0001**
day 7	1,51	1,68	1,43	1,65	1,47	2,71	0,40	**0,0666**	**0,0001**	NS	NS	NS	NS	**0,0001**	**0,0001**
day 0-7	0,37	0,42	0,69	0,35	0,50	0,57	0,38	NS[3]	NS	NS	NS	NS	NS	NS	NS
TBARS (mg MDA/g)															
0 min	1,65	1,26	1,28	1,46	1,29	0,87	0,37	**0,0036**	**0,0004**	NS	NS	NS	**0,0229**	**0,0022**	**0,0001**
30 min	1,62	1,23	1,34	1,44	1,27	0,83	0,33	**0,0020**	**0,0001**	NS	NS	NS	**0,0201**	**0,0002**	**0,0001**
60 min	1,71	1,53	1,44	1,58	1,46	1,03	0,40	**0,0401**	**0,0010**	NS	NS	NS	NS	**0,0028**	**0,0007**
90 min	1,93	1,65	1,55	1,81	1,53	1,14	0,35	**0,0036**	**0,0001**	NS	NS	NS	**0,0304**	**0,0004**	**0,0001**
120 min	1,91	1,81	1,77	1,98	1,82	1,17	0,41	NS	**0,0001**	NS	NS	NS	NS	**0,0001**	**0,0003**
Muscle glutathione (µM/g)															
GSSH	1,47	1,03	0,86	1,25	0,51	0,41	0,99	**0,075**	**0,094**	NS	NS	NS	NS	NS	**0,027**
GSHfree	34,00	35,51	37,02	29,09	33,29	32,11	8,04	NS	NS	**0,064**	NS	NS	NS	NS	NS
GSSH/GSH	4,22	2,80	2,63	5,02	1,54	1,29	0,006	NS	**0,096**	NS	**0,089**	NS	NS	NS	**0,054**

[1] RMSE: Root of the mean square error

[2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when $P < 0.05$

[3] NS: Not statistically significant

Table 5.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on colour (CIELAB L*, a*, b*, chroma and hue values) of muscle samples from pigs fed the experimental diets

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
L*															
Day 0	55,22	53,82	54,81	54,59	54,92	54,34	2,31	NS[3]	NS	NS	NS	NS	NS	NS	NS
Day 4	55,36	54,87	55,86	55,28	55,58	54,63	2,50	NS	NS	NS	NS	NS	NS	NS	NS
Day 8	56,07	55,20	56,22	55,29	56,18	55,78	2,83	NS	NS	NS	NS	NS	NS	NS	NS
a *															
Day 0	0,60	0,93	0,11	0,66	0,55	1,32	1,17	NS	0,0858	NS	NS	NS	NS	**0,0894**	NS
Day 4	3,40	3,35	2,84	2,94	2,90	4,02	1,17	NS	**0,0326**	NS	NS	NS	NS	**0,0236**	NS
Day 8	2,03	1,43	1,09	1,64	1,78	2,45	1,21	NS	**0,0597**	NS	NS	NS	NS	**0,0385**	NS
b*															
Day 0	12,10	11,45	11,43	11,74	11,83	12,21	0,91	NS	NS	NS	NS	NS	NS	**0,0854**	NS
Day 4	12,02	11,81	11,63	11,39	11,59	12,42	0,98	NS	**0,0484**	NS	NS	NS	NS	**0,0317**	NS
Day 8	11,88	11,31	11,38	11,46	11,20	11,27	1,07	NS	NS	NS	NS	NS	NS	NS	NS
Chroma															
Day 0	12,21	11,53	11,53	11,84	11,87	12,31	0,95	NS	NS	NS	NS	NS	NS	**0,0875**	NS
Day 4	12,57	12,31	12,02	11,82	11,98	13,09	1,21	NS	**0,0372**	NS	NS	NS	NS	**0,0235**	NS
Day 8	12,09	11,45	11,51	11,68	11,36	11,57	1,21	NS	NS	NS	NS	NS	NS	NS	NS
Hue															
Day 0	87,74	85,60	89,89	87,13	87,38	83,98	5,45	NS	**0,0792**	NS	NS	NS	NS	**0,0894**	NS
Day 4	74,94	74,42	76,73	76,05	76,03	72,30	4,45	NS	**0,0455**	NS	NS	NS	NS	**0,0395**	NS
Day 8	80,66	83,23	84,91	82,42	81,05	78,13	5,16	NS	**0,0262**	NS	NS	NS	NS	**0,0166**	NS

[1] RMSE: Root of the mean square error; [2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when $P < 0.05$; [3] NS: Not statistically significant

Table 6.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on pigment stability of muscle samples from pigs fed the experimental diets

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
Oxymyoglobin (OxyMb)															
Day 0	60,18	59,73	62,82	60,55	60,23	57,93	4,34	NS[3]	**0,0861**	NS	NS	NS	NS	**0,0786**	NS
Day 4	54,17	54,36	56,48	56,44	56,04	51,97	4,50	NS	**0,0368**	NS	NS	NS	NS	**0,0257**	NS
Day 8	59,14	61,66	64,61	63,26	62,22	60,51	5,34	0,096	NS	NS	NS	NS	**0,0625**	NS	NS
Deoxymyoglobin (DeoxyMb)															
Day 0	69,86	69,83	69,17	71,03	69,78	71,48	1,98	NS	**0,0378**	NS	NS	NS	NS	**0,0439**	**0,0899**
Day 4	91,15	90,33	90,86	90,80	90,28	91,63	1,22	NS	**0,0394**	NS	NS	NS	NS	**0,0239**	NS
Day 8	83,66	80,33	81,46	84,07	84,63	86,70	5,41	NS	**0,0562**	**0,0611**	NS	NS	NS	**0,0490**	NS
Metmyoglobin (MetMb)															
Day 0	135,87	135,84	135,04	135,49	135,95	135,77	2,14	NS	NS	NS	NS	NS	NS	NS	NS
Day 4	118,48	117,98	115,74	116,63	116,61	117,63	3,97	NS	NS	NS	NS	NS	NS	NS	NS
Day 8	114,38	117,70	108,70	107,07	107,63	105,08	9,38	0,140	**0,0853**	**0,0673**	NS	0,133	NS	NS	**0,0407**
MetMb / DeoxyMb															
Day 8	1,37	1,47	1,33	1,27	1,27	1,21	0,18	NS	**0,0434**	**0,0288**	NS	NS	NS	**0,0585**	**0,0625**

[1] RMSE: Root of the mean square error

[2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when $P < 0.05$

[3] NS: Not statistically significant

Table 7.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on free non-essential amino acids (mg/100 g muscle) at 2 or 8 days of meat storage from pigs fed the experimental diets.

								Probability of contrast [2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
SER day2	5.75	4.73	5.16	5.00	5.68	4.25	0.99	**0.0453**	**0.0112**	NS	NS	NS	NS	**0.0273**	**0.0040**
SER day7	9.04	9.29	9.81	9.43	10.48	7.91	2.02	NS[3]	**0.0355**	NS	NS	NS	NS	**0.0260**	NS
ASN day2	2.41	2.14	2.36	2.20	2.54	2.15	0.43	NS	NS	NS	**0.0709**	NS	NS	NS	NS
ASN day7	3.77	3.88	4.16	3.93	4.34	3.47	0.78	NS	**0.0778**	NS	NS	NS	NS	**0.0554**	NS
GLY day2	8.72	7.78	7.93	8.25	9.34	7.86	1.32	NS	NS	**0.0509**	NS	NS	NS	NS	NS
GLY day7	8.86	9.04	9.76	9.57	10.09	8.91	1.47	NS	NS	NS	NS	NS	NS	NS	NS
GLN day2	42.11	41.12	40.75	42.49	46.70	37.06	8.95	NS	NS	NS	NS	NS	NS	NS	NS
GLN day7	41.04	43.94	45.15	45.94	47.78	41.46	7.89	NS	NS	NS	NS	NS	NS	NS	NS
β-ALA day2	6.87	6.90	6.67	8.46	7.46	5.98	2.23	NS	NS	NS	NS	NS	NS	NS	NS
β-ALA day7	5.15	5.69	5.83	6.02	5.16	4.66	1.74	NS	NS	NS	NS	NS	NS	NS	NS
TAU day2	29.63	30.75	28.98	33.45	35.67	30.24	7.86	NS	NS	**0.0989**	NS	NS	NS	NS	NS
TAU day7	25.03	25.98	26.79	30.74	28.58	27.66	6.65	NS	NS	NS	NS	NS	NS	NS	NS
ALA day2	19.95	16.92	18.20	18.09	19.66	17.53	3.32	NS	NS	NS	NS	NS	NS	NS	NS
ALA day7	20.17	19.71	22.43	22.04	23.92	22.01	3.00	NS	NS	**0.0789**	**0.0358**	NS	NS	NS	NS
CAR day2	628.03	560.63	550.53	581.16	688.65	501.58	94.60	NS	**0.0091**	**0.0224**	NS	**0.0860**	NS	**0.0162**	**0.0106**
CAR day7	515.03	543.24	569.80	551.60	567.67	481.03	105.89	NS	NS	NS	NS	NS	NS	**0.0727**	NS
ANS day 2	26.57	26.85	25.53	28.69	30.25	20.93	4.62	NS	**0.0006**	**0.0512**	NS	NS	NS	**0.0005**	**0.0190**
ANS day7	22.41	24.51	23.81	25.11	24.30	19.76	4.50	NS	**0.0185**	NS	NS	NS	NS	**0.0119**	NS
ORN day2	0.52	0.53	0.47	0.36	0.40	0.42	0.13	NS	NS	**0.017**	NS	NS	NS	NS	NS
ORN day7	0.46	0.42	0.39	0.29	0.38	0.45	0.13	NS	NS	NS	NS	NS	**0.0867**	NS	NS

[1] RMSE: Root of the mean square error; [2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when P <0.05; [3] NS: Not statistically significant

Table 8.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on free essential amino acids (mg/100 g muscle) at 2 or 8 days of meat storage from pigs fed the experimental diets.

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
HIS day2	2.45	2.57	2.28	2.44	2.72	2.18	0.69	NS	NS	NS	NS	NS	NS	NS	NS
HIS day7	3.64	3.52	3.38	3.78	3.81	3.17	0.73	NS	NS	NS	NS	NS	NS	NS	NS
THR day2	3.86	3.48	3.99	3.42	4.34	4.03	0.95	NS	NS	NS	NS	NS	NS	NS	NS
THR day7	6.08	6.16	6.20	6.50	7.15	6.26	1.36	NS	NS	NS	NS	NS	NS	NS	NS
PRO day2	3.49	3.13	3.49	2.88	3.25	4.82	0.85	NS	**0.0001**	NS	NS	NS	NS	**0.0001**	**0.0032**
PRO day7	3.70	3.57	4.06	3.55	3.94	5.64	0.97	NS	**0.0001**	NS	NS	NS	NS	**0.0001**	**0.0003**
TYR day2	4.41	3.77	4.04	3.42	3.89	4.47	0.96	NS	NS	NS	NS	NS	0.164	**0.0764**	NS
TYR day7	6.85	6.79	7.04	6.54	6.62	7.67	1.30	NS	**0.0819**	NS	NS	NS	NS	**0.0815**	NS
VAL day2	4.17	3.52	4.03	3.43	3.63	3.71	0.73	**0.0830**	NS	NS	NS	NS	**0.0819**	NS	NS
VAL day7	5.50	5.49	6.12	5.60	5.91	5.74	0.88	NS	NS	NS	NS	NS	NS	NS	NS
MET day2	2.36	1.96	2.42	1.99	2.03	2.11	0.55	NS	NS	NS	NS	NS	NS	NS	NS
MET day7	4.48	4.48	4.97	4.59	4.78	4.61	0.85	NS	NS	NS	NS	NS	NS	NS	NS
ILE day2	3.67	2.88	3.67	2.88	3.08	2.88	0.60	**0.0139**	NS	NS	0.025	NS	**0.0260**	NS	**0.0115**
ILE day7	5.22	4.91	5.49	4.92	5.03	4.69	0.87	NS	NS	NS	NS	NS	NS	NS	NS
LEU day2	4.98	4.24	5.15	4.08	4.05	4.25	1.17	NS	NS	NS	NS	NS	NS	NS	NS
LEU day7	7.77	7.76	8.53	7.75	8.13	8.25	1.54	NS	NS	NS	NS	NS	NS	NS	NS
PHE day2	3.78	3.38	3.92	3.21	3.35	4.54	0.91	NS	**0.0062**	NS	NS	NS	NS	**0.0045**	NS
PHE day7	5.70	5.64	6.07	5.48	5.60	7.52	1.23	NS	**0.0004**	NS	NS	NS	NS	**0.0006**	**0.0052**
TRP day2	1.05	1.00	1.02	0.86	0.93	1.29	0.28	NS	**0.0063**	NS	NS	NS	NS	**0.0046**	NS
TRP day7	1.29	1.35	1.37	1.22	1.22	1.89	0.36	NS	**0.0001**	NS	NS	NS	NS	**0.0001**	**0.0018**
LYS day2	3.60	3.21	3.56	2.65	3.14	3.03	0.86	NS	NS	NS	NS	NS	NS	NS	NS
LYS day7	5.39	5.43	5.64	4.25	4.78	5.72	1.50	NS	NS	0.061	NS	NS	NS	NS	NS

[1] RMSE: Root of the mean square error; [2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS.. Differences were statistically significant when $P < 0.05$; [3] NS: Not statistically significant

Table 9.- Effect of selenium source (organic, SY vs. mineral, SS), dose (low, L vs. high, H) and vitamin E supplementation on myofibrillar fragmentation index (MFI) from pigs fed the experimental diets.

								Probability of contrast[2]							
	CONTROL	SS-L	SS-H	SY-L	SY-H	VITE+SS	RMSE[1]	1	2	3	4	5	6	7	8
MFI															
Day 0	28.41	26.44	25.34	30.51	29.42	29.33	10.55	NS[3]	NS	NS	NS	NS	NS	NS	NS
Day 8	64.16	57.86	55.93	61.89	78.52	78.76	21.21	NS	**NS**	**NS**	NS	NS	NS	**NS**	NS

[1] RMSE: Root of the mean square error; [2] Probability of contrast: (1): CONTROL vs others; (2): VITE+SS vs others; (3): SeY vs SS; (4) S-L vs S-H; (5): Se source x Se dose interaction; (6) CONTROL vs. Se; (7) VITE+SS vs Se; (8) CONTROL vs. VITE+SS. Differences were statistically significant when $P < 0.05$

[3] NS: Not statistically significant

Highlights

- The influence of selenium source and dose on muscle proteolytic activity was studied.
- Organic Se effectively increased WHC and post-mortem muscle proteolytic activity.
- Dietary vitamin E+Se supplementation was not as effective as organic Se in improving WHC. These results would confirm the relation between muscle proteolysis and WHC.