



Effects of increasing dietary organic selenium levels on meat fatty acid composition and oxidative stability in growing rabbits



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ABSTRACT

The effects of dietary organic selenium (Se) addition at 0.1, 0.5 and 2.5 mg/kg vs. an unsupplemented basal diet (BD) on performance, fatty acid (FA) composition and oxidative stability were studied in muscle tissue of growing rabbits. Muscle Se content increased ($P < 0.001$) in a dose dependent manner with dietary Se inclusion. Saturated FA (SFA) were affected linearly ($P < 0.05$) and quadratically ($P < 0.05$) by dietary Se addition. Polyunsaturated FA (PUFA) increased linearly ($P < 0.01$) resulting in a linear increase in the PUFA:SFA ratio ($P < 0.01$) with dietary Se increment. Feeding 0.5 mg Se/kg diet reduced malondialdehyde (MDA) and oxygen radical absorbance capacity (ORAC) values in the muscle, whilst 2.5 mg Se/kg diet increased MDA concentrations and tended to increase ORAC values, likely indicating oxidative stress. In conclusion, dietary Se supplementation at 0.5 mg/kg improves meat FA composition and oxidative stability, whereas at 2.5 mg/kg may induce pro-oxidant effects.

1. Introduction

Rabbit meat exhibits excellent nutritive and dietetic properties (Combes & Dalle Zotte, 2005; Hernández & Gondret, 2006) among which, the high content in polyunsaturated, particularly *n*-3, fatty acids (FA) has gained momentum (Dalle Zotte & Szendrő, 2011). However, *n*-3 FA is prone to oxidation resulting in reduced meat and meat product shelf-life (Wood et al., 2004) and undesired flavour development (Gandemer, 1998). To this aspect, dietary supplementation with various antioxidants has become a useful tool to improve oxidative stability and shelf life of rabbit meat.

The key dietary factors of antioxidant protection are vitamin E and selenium (Se). Vitamin E has been adequately investigated in rabbits and many studies have confirmed that feeding supra-nutritional levels of α -tocopheryl acetate (100–375 mg/kg feed), improves the oxidative stability of raw, cooked and refrigerated or frozen rabbit meat (Castellini, Dal Bosco, & Bernardini, 1999; Castellini, Dal Bosco, Bernardini, & Cyril, 1998; Dal Bosco, Castellini, & Bernardini, 2001; Ebeid, Zeweil, Basyony, Dosoky, & Badry, 2013; Lo Fiego et al., 2004; Oriani et al., 2001), reducing the loss of long chain *n*-3 FA (Bernardini, Dal Bosco, & Castellini, 1999; Castellini et al., 1999; Dal Bosco et al., 2001), and consequently improving its nutritional value. The trace mineral Se is an integral part of at least 25 selenoproteins, some of which (glutathione peroxidase-GSHPx and thioredoxin reductase) are involved in cellular antioxidant defense and redox regulation (Dalle

Zotte & Szendrő, 2011; Pappas, Zoidis, Surai, & Zervas, 2008). Animals can readily incorporate Se into edible tissues so that it is possible to produce Se-enriched meat (Surai, 2006) with improved oxidative stability, when feeding Se supplements. Studies in broilers have showed that supranutritional Se levels (3 mg/kg) resulted in higher meat Se content, higher PUFA levels and improved oxidative stability (Pappas, Zoidis, Papadomichelakis, & Fegeros, 2011). In contrast, information on the effect of dietary Se supplementation in rabbits is limited. The few studies have shown that meat Se levels are readily elevated from 9.3 to 15.0 $\mu\text{g}/100\text{ g}$ in non-supplemented diets to about 24–39.5 $\mu\text{g}/100\text{ g}$ following the supplementation of 0.40–0.50 mg of Se-yeast or Se-algae/kg feed (Dokoupilová, Marounek, Skřivanová, & Březina, 2007; Marounek, Dokoupilová, Volek, & Hoza, 2009); however, no corresponding effects on meat oxidative stability were observed (Marounek et al., 2009). Both Dokoupilová et al. (2007) and Marounek et al. (2009) concluded that the only benefit of dietary Se supplementation was the enrichment of meat with Se. More recent studies observed that dietary supplementation with sodium selenite at 0.41–0.59 mg/kg feed (Zhang, Zhu, Wang, Wang, & Li, 2011) or Se-yeast at 0.3 mg/kg feed (Ebeid et al., 2013) may significantly retard oxidation in liver and meat, respectively, in addition to tissue enrichment with Se. There is no clear recommendation for the appropriate amount of supplemental Se in growing rabbits. According to NRC (1977) and de Blas and Mateos (1998), the minimum Se requirements in rabbits for a normal growth rate are quite low, but a small amount of additional Se (0.05 mg/kg

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feed) is advisable, due to its potential effects on other physiological functions, as a constituent of enzyme complexes (Mateos, Rebollar, & de Blas, 2010), mainly selenoproteins.

The up to date work in rabbits has focused on the investigation of the effects of dietary Se (either organic or inorganic) supplementation, at levels lower than 0.70 mg/kg, on growth, GSH-Px activity and expression, and lipid oxidation in meat, liver and blood. There is no data available to our knowledge, on the effects of dietary Se levels higher than 0.70 mg/kg, and moreover, the potential impact of such levels on growth, meat FA composition and oxidative stability is not yet known. Therefore, the present study sought to investigate whether dietary organic Se addition, at levels similar to those used in previous studies, or well above those, may affect performance, meat FA profile and oxidative stability in growing rabbits.

2. Materials and methods

2.1. Animals and diets

Ninety-six healthy 35-day-old weaned Hyla hybrid male animals were purchased from a breeding farm for meat rabbits. Upon arrival at the facilities of the Department of Nutritional Physiology and Feeding, they were randomly allocated into four groups, namely control (BD), LSe, MSe and HSe, of 24 rabbits each. They were kept indoors under natural environmental conditions in individual wire mesh cages equipped with metal troughs and automatic nipple drinkers. The rabbits of control group were fed a basal pelleted diet (BD), without any Se supplementation; the only Se present was that of feed ingredients (Table 1). The LSe, MSe and HSe rabbits were fed the same basal diet supplemented with Se from a yeast source, Sel-Plex® (Alltech Inc., Nicholasville, KY, USA), to provide additional 0.1, 0.5 and 2.5 mg Se/kg of diet, respectively. The BD was formulated according to the recommendations of de Blas and Mateos (1998) for growing rabbits. The ingredients and chemical composition of the diets are shown in Table 1.

2.2. Experimental procedures

Handling and care of the experimental animals conformed to the guidelines of the Faculty of Animal Science and Aquaculture. During the experiment, body weight and feed intake were recorded weekly. At the end of the experiment (77 days of age) rabbits were sacrificed by cervical dislocation, eviscerated and after a 24 h chilling period at 4 °C, carcasses were weighed and dressing percentage was determined. Subsequently, samples from the *Longissimus lumborum* (LL) muscle were collected. In detail, from each carcass, the right part of the LL muscle was excised, vacuum packed and stored at –0 °C until analyzed for FAs. A smaller portion (ca. 2–3 g) from the right part of the LL muscle was used for the determination of Se concentration. Simultaneously, the left part of the muscle was removed, vacuum packed and stored at –20 °C, so as to determine lipid oxidation and total antioxidant capacity using the oxygen radical absorbance capacity.

2.3. Determination of selenium in diets and muscle tissue

Selenium concentration in feed and LL samples was determined using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, Elan 9000; PerkinElmer Life and Analytical Sciences Inc., Waltham, MA, USA). Feed samples were collected prior to feeding and milled prior to analysis through a 1-mm sieve (Cyclotec, 1093 sample mill; Tecator, Höganäs, Sweden). For total Se determination, complete digestion of the samples was performed with a microwave digestion system (Mars X-Press; CEM, NC, USA). Samples (0.5 g) of wet tissue or feed were soaked in 10 ml concentrated HNO₃ (65% w/v, Suprapur; Merck, Darmstadt, Germany). The samples were heated in the microwave-accelerated digestion system according to the following

Table 1
Ingredient and chemical composition of the basal diet (g/kg as fed basis), and selenium (Se) level of the experimental diets.

		Basal diet
Ingredient		
Dehydrated alfalfa		294.0
Barley grain		170.0
Wheat bran		284.0
Sunflower meal, 280 g CP/kg		154.0
Citrus pulp		80.0
L-lysine HCl, 80%		2.6
DL-methionine, 99%		2.3
L-threonine		2.0
Sodium chloride		4.1
Ultrafed ^{a,b}		3.5
Premix ^b		3.5
Calculated chemical composition		
Dry matter		892.0
Organic matter		931.7
Crude protein		164.2
Ether extract		22.0
NDF		333.0
ADF		187.9
Lysine		7.8
Methionine + cystine		7.5
Threonine		7.8
Calcium		9.6
Phosphorus		6.0
Digestible energy, MJ/kg ^c		9.9
Diet		
	Se level (mg/kg)	
	Added ^d	Determined ^e
Basal (control)	–	0.068 ± 0.018
LSe	0.1	0.160 ± 0.010
MSe	0.5	0.519 ± 0.060
HSe	2.5	1.949 ± 0.108

^a Contained > 95% palygorskite [(Mg,Al)₂Si₄O₁₀(OH)₄(H₂O)] as agglomerant (binder).

^b Premix provided per kg diet: vitamin A, 10,000 IU; vitamin D3, 1800 IU; vitamin E, 60 IU; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 0.02 mg; calcium pantothenate, 7 mg; nicotinic acid, 30 mg; folic acid, 0.5 mg; biotine, 0.2 mg; choline chloride, 400 mg; I, 1.5 mg; Mn, 60 mg; Cu, 6 mg; Zn, 80 mg; Fe, 30 mg; Co, 0.35 mg; antioxidant, 0.250 mg; 300 mg Cycostat (60 mg robenidine/kg). It did not contain any Se source (organic or inorganic).

^c From tabulated data (FEDNA, 2003).

^d Se was added in the form of Sel-Plex® (Alltech Inc., Nicholasville, KY, USA) and only in the Se supplemented diets (LSe, MSe and HSe).

^e Average of 4 samples per diet ± standard deviation.

programme: the power was ramped during 20 min from 100 to 1200 W and held for 15 min. The temperature reached a maximum of 200 °C and followed by a cool down cycle for 15 min. Losses of volatile element compounds do not occur as the tubes are sealed during heating. The samples were then filtered with disposable syringe filters (Chromafil; Macherey-Nagel, Duren, Germany) and diluted 50 times with reversed osmosis water (Milli-Q Water Purification Systems, Billerica, MA, USA) prior to injection in the ICP-MS instrument. Standard solutions used for calibration curves were prepared from high-purity standards (Multielement standard solution, Fluka Analytical; Sigma-Aldrich, St Louis, USA). The method and the instrumental parameters of the equipment have been described previously (Zoidis, Pappas, Georgiou, Komaitis, & Fegeros, 2010). In brief, the instrumental parameters of the equipment used were as follows: the nebulizer flow was 0.775 L/min, the vacuum pressure was 1.5×10^{-5} Torr, the lens voltage was 950 V, the analogue stage voltage was 1900 V whilst the pulse stage voltage was 950 V. Twenty sweeps per reading were performed with 1 reading per replicate. The total number of replicates was 3. The total time per sample was 83 s. The analytical procedure was validated using standard reference

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