World Rabbit Science

World Rabbit Sci. 2014, 22: 113-121
doi:10.4995/wrs.2014.1760


# EFFECT OF HESPERIDIN DIETARY SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS TRAITS AND MEAT QUALITY OF RABBITS

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Abstract: An experiment was conducted to examine the dose effects of hesperidin dietary supplementation on fattening rabbits' growth performance, as well as carcass and meat quality characteristics. Forty-eight Hyla hybrid male weaned (35 d old) rabbits were purchased and randomly assigned to 3 dietary groups of 16 rabbits each and fed diets supplemented with the antioxidant hesperidin at 0, 1 and 2 g/kg feed. At 80 d of age, the rabbits were slaughtered and samples of *Longissimus lumborum* (*LL*) muscle were used to estimate meat quality traits. No significant differences were observed in body weight at the age of 80 d, feed conversion rate (35 to 80 d), or organ weights among the 3 groups. The pH, colour, percentage of released water, shear force values and intramuscular fat content of LL muscle were not significantly influenced by the dietary treatment. Hesperidin dietary supplementation at both levels reduced the polyunsaturated fatty acids (PUFAs), mainly arachidonic (C20:4n-6), docosapentaenoic (C22:5n-3) and eicosapentaenoic (C20:5n-3) (only at 2 g/kg), and PUFA/SFA ratio ($P<0.01$). Based on the malondialdehyde (MDA) values, hesperidin inclusion did not influence meat antioxidant status during the 9-d refrigerated storage at 4°C. Thus, we may conclude that dietary supplementation with hesperidin at the selected concentration levels did not generally influence growth performance, carcass traits, meat quality or antioxidant capacity in fattening rabbits, although meat values for PUFAs appeared to be decreased.

Key Words: hesperidin, rabbit growth, oxidation, carcass, fatty acids.

## INTRODUCTION

The meat quality concept is highly correlated with its healthiness, hedonistic quality, sensory properties, cooking ease and swiftness, as well as price (Dalle Zotte, 2002). Much attention has recently been paid to the development of habitually consumed products with physiological functions that promote human health and reduce the prevalence of chronic diseases, such as cardiovascular diseases (coronary heart disease, atherosclerosis) and some forms of cancer (breast, prostate, pancreas, oesophagus, stomach and colon, etc.) (Bellisle *et al.*, 1998).

Rabbit meat possesses high nutritional and dietetic properties, as it is lean and the lipids are highly unsaturated (60% of total fatty acids). It is rich in proteins (20-21%), its amino acids are of high biological value and its concentration in cholesterol is low (Dalle Zotte and Szendrő, 2011). Content in polyunsaturated fatty acids (PUFA) is relatively high, but the n-6 PUFA/n-3 PUFA ratio is very low (Alasnier and Gandemer, 1998; Castellini *et al.*, 1998; Petracci and Cavani, 2013). However, increasing the degree of unsaturation of animal tissues accelerates oxidative deterioration by free radicals during meat processing and storage (Kanner, 1994).

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http://dx.doi.org/10.4995/wrs.2014.1760

Consumption of oxidised meat products may compromise human health. Prevention of lipid oxidation in muscle-based food can be achieved by the addition of antioxidants. Over the last decade, considerable interest has arisen in the use of natural antioxidants that would serve as alternatives to synthetic supplements in order to improve meat quality, without leaving residues in the product or the environment (Yanishlieva-Maslarova, 2001). As a result, there is a strong tendency towards isolating organic antioxidants from natural sources for the protection of animal health and products against oxidation (Wenk, 2003). The use of natural antioxidants can prolong the shelf life and increase the acceptability of meat and its economic value in the marketplace. Nutritional approaches are often more effective than direct addition of the additive to the muscle food, since the compound is preferably deposited where it is most needed (Govaris *et al.*, 2004). Antioxidant effects of α-tocopheryl acetate (vitamin E) supplementation on rabbit muscles are well established (Castellini *et al.*, 1998; Corino *et al.*, 1999).

The antioxidant properties of citrus by-products have increased interest in their use as alternative feeds for animals. Dried citrus pulp is the main by-product from the citrus-processing industry and produced after extraction of the juice from citrus fruits and drying out the residues. Citrus pulp is a mixture of peel, inside portions and culled fruits. Fibres from citrus fruits have an additional advantage over dietary fibres from other sources due to the presence of associated bioactive compounds (i.e. flavonoids). These compounds usually contain one or more aromatic hydroxyl groups, which actively scavenge free radicals and are responsible for the antioxidant activity. At the same time, citrus pulp constitutes a cheap feed, particularly during the dry summer when grass land is very limited in countries around the Mediterranean and reduces the dependence of livestock on grains that can be consumed by humans (Martínez-Pascual and Fernández-Carmona, 1980; Bampidis and Robinson, 2006).

Hesperidin, a bioflavonoid, is an abundant and inexpensive by-product of citrus cultivation. It is a naturally occurring polyphenolic compound widely distributed in the plant kingdom as a secondary metabolite. Hesperidin is a flavanone glycoside comprising an aglycone, hesperitin or methyl eriodictyol and an attached disaccharide, rutinose (Garg *et al.*, 2001). Pure hesperidin occurs as long hair-like needles, tan or pale yellow in colour. It is tasteless and odourless. A deficiency of this substance in human diet has been linked with abnormal capillary leakage as well as pain in the extremities causing aches, weakness and leg cramps (Garg *et al.*, 2001).

Hesperidin has been reported to possess *in vitro* antioxidant activity and radical scavenging properties (Garg *et al.*, 2001). On the other hand, there are no reports on the potential effects of hesperidin dietary supplementation in rabbits. The aim of the present study was therefore the evaluation of hesperidin dietary supplementation dose effects on growth parameters, carcass traits and meat quality in fattening rabbits.

## MATERIALS AND METHODS

### *Animals and Diets*

Forty-eight Hyla hybrid male weaned (35 d old) rabbits were purchased and randomly assigned to 3 dietary groups of 16 rabbits each and fed diets supplemented with pure hesperidin (MP Biomedicals, LLC, Illkirch, France) at 0, 1 and 2 g/kg feed. Diet was formulated according to the recommendations of De Blas and Mateos (1998) for fattening rabbits. The ingredients and chemical composition of the diet (without hesperidin supplementation) are shown in Table 1. The levels of supplementation were chosen to reach the maximum levels of hesperidin ingestion by the rabbit when consuming a diet supplemented with approximately 50% citrus pulp. Citrus pulp contains hesperidin at a level of 5 g/kg (Antongiovanni *et al.*, 2005) and its dietary supplementation at a level of 45% does not influence rabbit performance and meat quality, according to previous experiments (Martínez-Pascual and Fernández-Carmona, 1980).

Rabbits were kept indoors in individual cages (0.41×0.33×0.29 m) with wire mesh floors, under controlled environmental conditions (temperature: 22.5±3.5 ºC; relative humidity: 50±20%; lightning: 12/12 h light/dark cycle). Each cage was equipped with a metal feeder and an automatic nipple drinker. Feed was provided *ad libitum* and rabbits had free access to water. The methods used in the present experiment were in accordance with the national legislation and the guidelines of the Research Ethics Committee of the Department of Animal Science and Aquaculture of the Agricultural University of Athens.

Table 1: Ingredients and chemical composition of the basal (control) diet (g/kg).

| Ingredients | |
|---|---|
| Dehydrated alfalfa meal, 15-17% CP | 260 |
| Dried beet pulp | 140 |
| Wheat bran | 180 |
| Barley | 272 |
| Soybean meal, 45% CP | 35 |
| Sunflower meal | 100 |
| Limestone | 4 |
| Salt (NaCl) | 4.5 |
| L-lysine HCl, 80% | 2 |
| DL-methionine, 99% | 2 |
| L-threonine | 2.5 |
| Mineral/vitamin premix[a] | 5 |
| Calculated chemical composition | |
| Digestible energy (MJ/kg) [b] | 9.97 |
| Dry matter | 900 |
| Crude protein | 161.3 |
| Ether extract | 19.7 |
| Crude fibre | 154 |
| Neutral Detergent Fibre | 323 |
| Acid Detergent Fibre | 183 |
| Acid Detergent Lignin | 36 |
| Calcium | 8 |
| Phosphorus | 5 |
| Sodium | 2 |
| Lysine | 8.5 |
| Methionine+cystine | 7.4 |
| Threonine | 8.6 |

[a]Mineral and vitamin premix, provided per kg of diet: vitamin A, 9000 IU; vitamin $D_3$, 1800 UI; vitamin E, 40 UI; vitamin $K_3$, 1 mg; vitamin $B_2$, 2 mg; vitamin $B_{12}$, 0.01 mg; pantothenic acid, 1 mg; nicotinic acid, 15 mg; choline, 338 mg; I, 1.3 mg; Mn, 46.5 mg; Cu, 35 mg; Zn, 80 mg; Fe, 28 mg; Se, 0.01 mg; antioxidant (ethoxyquin), 0.250 mg; 300 mg cycostat (60 mg robenidine/kg).
[b] Calculated values (FEDNA, 2003).

Feed intake and body weight were weekly recorded to estimate body weight gain and feed conversion ratio. At day 80 of age, rabbits were weighed (final body weight; FBW), slaughtered and weights of liver, kidneys, thoracic organs, perirenal and pelvic fat were recorded. After refrigerated storage for 24 h at 4°C, *Longissimus lumborum (LL)* muscle was excised and used for the meat quality analyses.

### *$pH_{24}$ and meat colour*

pH was measured using a Sentron 1001 pH System (Roden, Netherlands) model, with the electrode inserted into the *LL* muscle 24 h after slaughter. pH meter calibrated in buffers at pH 4.0 and 7.0 (Merck, Darmstadt, Germany) at ambient temperature. For colour measurements, *LL* muscle was sliced across the fibres, left exposed to the air at room temperature for blooming during 30 min and measured (3 measurements per sample) using a Miniscan XE Chroma Meter (HunterLab, Reston, VA, USA) set on the L*, a*, b* system (CIE 1976, Commission International de l'Eclairage). A white and a black tile were used as standards.

### *Shear force and cooking loss*

A sample of *LL* muscle from each rabbit was weighed, placed in plastic bag and cooked in a water bath at 80 ˚C for 60 min, left under running water for 15 min and then placed in room temperature. The sample was weighed again to estimate the percentage of cooking loss (%). Three sub samples with a cross section of 1 $cm^2$ were then cut parallel to the muscle fibres and shear force value of the *LL* muscle was measured using a Warner Bratzler shear blade fitted

to a Zwick Testing Machine Model Z2.5/TN1S (Zwick GmbH & Co, Ulm, Germany). Peak force values in Newton were recorded.

### *Percentage of released water*

In duplicate, muscle samples weighing about 300 mg (P1) were placed between 2 pieces of filter paper, and pressed for 5 min, using a weight of 2.25 kg. The muscle samples were then removed and re-weighed (P2). Percentage of released water was determined as PRW (%)=(P1–P2)/P1×100.

### *Measurement of total lipids and lipid oxidation – MDA assay*

Measurement of intramuscular total lipids was performed using the method first described by Folch *et al.* (1957). Tissue samples were homogenised with 2:1 chloroform-methanol mixture to a final dilution 20-fold the volume of the tissue sample. The crude extract was mixed with 0.2 of its volume of water and separated into 2 phases. The lower phase contained the tissue lipids.

Lipid oxidation was assessed on the basis of the malondialdehyde (MDA) formed during storage, a secondary lipid oxidation product formed by hydrolysis of lipid hydroperoxides. In the present study, MDA concentration in *LL* muscle samples was determined 1, 3, 6 and 9 d after storage at 4 ºC, using a selective third-order derivative spectrophotometric method, previously developed by Botsoglou *et al.* (1994). Derivative *vs.* conventional spectrophotometry was adopted because it offers improved sensitivity, specificity and reliability of the measurements, as it eliminates potential interferences from other reactive compounds.

In brief, 2 g of each sample (2 samples per rabbit) were homogenised (Edmund Buehler 7400 Tuebingen/H04, Germany) in the presence of 8 mL aqueous trichloroacetic acid (50 g/L) and 5 mL butylated hydroxytoluene in hexane (8 g/L), and the mixture was centrifuged for 3 min at 3000 g. The top hexane layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL aqueous 2-thiobarbituric acid (8 g/L) to be further incubated at 70 ˚C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to third-order derivative spectrophotometry (Hitachi U3010 Spectrophotometer) in the range of 500-550 nm. The concentration of MDA (ng/g wet tissue) in analysed samples was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of standard calibration curve prepared using 1,1,3,3-tetraethoxypropane, the MDA precursor.

### *Determination of fatty acid profile*

Any external fat and connective tissue were dissected out of the *LL* muscle samples, which were then blended in a food processor until smooth. Blending was performed in short bursts to ensure the homogeneous distribution of intramuscular fat in the sample. Duplicate 1 g samples were hydrolysed for 1.5 h at 55°C in 1 N potassium hydroxide in methanol, containing a known amount (approx. 0.5 mg) of tridecanoic acid (C13:0) methyl ester as an internal standard. The potassium hydroxide was then neutralised and the free fatty acids were methylated by sulphuric acid catalysis (24 $NH_2SO_4$) for 1.5 h at 55°C. Hexane (3 mL) was added to the reaction tube, which was vortex-mixed and centrifuged. The supernatant hexane layer containing the fatty acid methyl esters was pipetted into a clean reaction tube and evaporated under a nitrogen stream at 55°C. Methyl esters were then rediluted in 0.5 mL hexane, transferred into gas chromatography vials and kept at –20°C. They were subsequently analysed by gas chromatography in a temperature-programmed run using a Perkin Elmer Autosystem XL gas chromatograph equipped with a 60 m×0.25 mm×0.20 µm internal diameter HP-88 capillary column (Agilent Technologies, J&W GC columns) and flame ionisation detector (FID). The oven temperature was programmed for 1 min at 140°C, raised by 4°C/min to 200°C, then to 230°C by 1°C/min and finally to 240°C by 4°C/min and held for 10 min. Helium was the carrier gas at a constant pressure of 18 psi and the temperature of both the injector and FID was set at 250°C. Fatty acids were identified by comparison with standards purchased from Sigma-Aldrich Co. (FAME 37 Component and BAME Mix, Supelco, USA). Quantification was achieved using the internal standard added prior to hydrolysis.

Total weights of fatty acids of *LL* muscles (mg/100 g wet muscle weight) were calculated as the sum of areas for all fatty acid peaks compared to area for 0.5 mg internal standard and were used to describe total lipids due to the method's precision (O'Fallon *et al.*, 2007). Individual fatty acids were expressed as % by weight of total fatty acids.

### *Statistical analysis*

FBW, feed conversion ratio, internal organ weights, carcass and meat quality characteristics as well as fatty acid profile were analysed using a Mixed Model procedure which contained the fixed effect of nutritional treatment. MDA concentration was analysed using a Mixed Model appropriate for repeated measurements per subject, which included the effect of nutritional treatment as fixed effect. All model analyses were performed by SAS/STAT (2005).

## RESULTS AND DISCUSSION

### *Growth performance*

Both levels of dietary hesperidin supplementation (1 and 2 g/kg feed) had no significant effect on the FBW and feed conversion ratio among the experimental groups (Table 2). The same result has also been reported by other researchers after the administration of antioxidants in fattening rabbits' diets, i.e. vitamin E (Castellini *et al.*, 1998) and vitamin C (Castellini *et al.*, 2000). Furthermore, supplementation of rabbits' diets with oats (Lopez-Bote *et al.*, 1998) or chia seed (Peiretti and Meineri, 2008), 2 feeds that have several compounds with potent antioxidant properties, appears not to affect feed efficiency and body weight. On the other hand, the addition of bee pollen (200 mg/kg feed), a supplement with well known antioxidant properties, resulted in a reduction of feed conversion ratio in growing rabbits (4-12 wk of age) from 5.64 to 4.23 (Attia *et al.*, 2011).

### *Carcass and internal organs*

The dressing percentage and the weights of various carcass parts and organs of rabbits are shown in Table 2. The incorporation of different levels of hesperidin in diet did not significantly influence the above parameters. Previous researchers reached the same conclusions after the inclusion of chia (*Salvia hispanica* L.; Peiretti and Meineri, 2008) or false flax (*Camelina sativa* L.; Peiretti *et al.*, 2007) in rabbit diets, as the use of the above seeds did not seem to affect the main carcass traits.

Table 2: Effect of dietary hesperidin supplementation on body weight, feed conversion ratio, carcass characteristics and internal organs' weights of fattening rabbits (least square means)[1].

| | Hesperidin supplementation (g/kg feed) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | SEM | *P*-value |
| Body weight at 35 d of age (kg) | 1.03 | 1.04 | 1.02 | 0.02 | 0.77 |
| Body weight at 80 d of age (FBW, kg) | 3.16 | 3.18 | 3.04 | 0.05 | 0.10 |
| Feed Conversion Ratio (35-80 d) | 3.36 | 3.50 | 3.55 | 0.07 | 0.17 |
| Cold Carcass Weight (CCW, kg) | 1.70 | 1.69 | 1.62 | 0.03 | 0.14 |
| Dressing percentage (%) | 53.7 | 53.2 | 53.3 | 0.51 | 0.76 |
| Head (% CCW) | 11.0 | 10.5 | 10.6 | 0.21 | 0.22 |
| Hind part weight (% CCW) | 33.7 | 33.5 | 33.9 | 0.40 | 0.73 |
| Fore part weight (% CCW) | 31.9 | 32.6 | 32.1 | 0.37 | 0.31 |
| Intermediate part weight (% CCW) | 23.7 | 24.0 | 24.3 | 0.37 | 0.50 |
| Liver (% FBW) | 3.60 | 3.67 | 3.38 | 0.13 | 0.26 |
| Lungs+Heart (% FBW) | 1.17 | 1.18 | 1.19 | 0.35 | 0.90 |
| Kidneys (% FBW) | 0.60 | 0.59 | 0.63 | 0.02 | 0.13 |
| Pelvic fat (% FBW) | 0.42 | 0.36 | 0.33 | 0.04 | 0.25 |
| Perirenal fat (% FBW) | 0.98 | 1.05 | 1.11 | 0.08 | 0.56 |

SEM: standard error of the means.

[1]16 animals per experimental group.

Table 3: Effect of hesperidin dietary supplementation on meat quality characteristics of 80 d old fattening rabbits (least square means)[1].

| | Hesperidin supplementation (g/kg feed) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | SEM | *P*-value |
| pH (24 h) | 5.53 | 5.52 | 5.53 | 0.01 | 0.93 |
| Colour | | | | | |
| L* | 52.3 | 51.8 | 52.1 | 0.38 | 0.72 |
| a* | 3.55 | 3.38 | 3.41 | 0.17 | 0.77 |
| b* | 9.44 | 9.78 | 9.56 | 0.13 | 0.22 |
| Released water (%) | 29.5 | 28.8 | 29.8 | 0.96 | 0.77 |
| Intramuscular fat (%) | 1.30 | 1.44 | 1.46 | 0.06 | 0.17 |
| Cooking loss (%) | 28.7 | 29.5 | 30.6 | 0.59 | 0.09 |
| Shear Force (N) | 20.9 | 21.7 | 22.6 | 0.98 | 0.48 |

SEM: standard error of the means.

[1]16 animals per experimental group.

### *Meat quality*

Different levels of dietary hesperidin supplementation appeared not to affect *LL* muscle quality characteristics, such as $pH_{24}$, colour parameters, percentage of released water, cooking loss and shear force values (Table 3). On the contrary, other researchers have found increased percentage of released water and reduced shear values in rabbit meat obtained from animals fed a diet supplemented with 200 mg of α-tocopherol acetate per kg feed (Castellini *et al.*, 1998). Moreover, dietary α-tocopherol acetate supplementation has been found to stabilise the surface colour of raw and cooked rabbit meat (Castellini *et al.*, 1998; Corino *et al.*, 1999).

As presented in Table 4, refrigerated storage increased the levels of MDA, the compound used as an index of lipid oxidation. However, results indicated that incorporation of hesperidin into the rabbits diets at the levels of 1 and 2 g/kg did not significantly reduce lipid oxidation in the refrigerated *LL* muscle samples. Hesperidin demonstrates a remarkable *in vitro* antioxidant activity (Garg *et al.*, 2001), so the reason for the lack of the same positive *in vivo* effect in rabbits could be due to the supplementation levels selected and/or possible interactions with the rabbit's digestive system and physiology.

Supplementing rabbit diets with adequate amounts of vitamin E (Corino *et al.*, 1999) or both vitamins E and C (Castellini *et al.*, 2000; Lo Fiego *et al.*, 2004) appears to significantly delay the oxidative processes and promote the lipid stability of rabbit meat during storage. In addition, dietary oregano essential oil exerted a significant antioxidant effect on rabbit meat at the level of 200 mg/kg (Botsoglou *et al.*, 2004). Moreover, thiobarbituric acid reactive

Table 4: Effect of the dietary hesperidin supplementation and the storage time on the lipid oxidation values (ng of malondialdehyde/g) in the raw rabbit *Longissimus lumborum* muscle (least square means)[1].

| | Hesperidin supplementation (g/kg feed) | | | | |
|---|---|---|---|---|---|
| Storage time at 4ºC (d) | 0 | 1 | 2 | SEM | *P*-value |
| 1 | 9.54[a] | 9.43[a] | 9.02[a] | 0.51 | 0.75 |
| 3 | 17.5[b] | 16.9[b] | 13.5[ab] | 1.41 | 0.13 |
| 6 | 37.5[b] | 37.0[b] | 36.2[b] | 6.39 | 0.99 |
| 9 | 49.1[b] | 48.7[b] | 48.3[b] | 7.31 | 0.99 |

Higher levels of malondialdehyde indicate higher rates of lipid oxidation. SEM: standard error of the means.

[1]16 animals per experimental group.

Storage time effect was significant ($P<0.001$) but the interaction of storage time with treatment was not significant ($P=0.9935$).

[a,b]Means in a column with different superscripts are significantly different at $P<0.05$.

substances of muscle samples from rabbits fed diets containing oats were decreased compared to those of the rabbits fed a control diet, as a result of the several phenolic compounds contained (Lopez-Bote *et al*., 1998). On the other hand, no effect of Spirulina dietary supplementation on oxidative stability of rabbit meat was found (Dal Bosco *et al*., 2014). These differences are possibly related with the different mechanisms of action (scavenger, chain-breaking etc) and the variation in the absorption from the gut as a result of the characteristics of the different antioxidants included in the vegetal essences.

### *Fatty acid profile*

The fatty acid composition of the *LL* muscle is presented in Table 5. The inclusion of hesperidin at both levels increased the percentage of myristic (C14:0) ($P<0.001$) and decreased the level of stearic (C18:0) ($P<0.001$); however, the total amount of SFA was not significantly different among groups and this finding was in line with previous experiments in rabbit (Bovera *et al*., 2012). Among MUFA, hesperidin supplementation at both levels increased the percentage of palmitoleic (C16:1n7) ($P<0.05$) but the total amount of MUFA was similar among the experimental groups. Hesperidin dietary supplementation reduced the PUFAs ($P<0.01$), mainly arachidonic (C20:4n-6), eicosapentaenoic (C20:5n-3) (only at 2 g/kg) and docosapentaenoic (C22:5n-3) ($P<0.01$). As a consequence, PUFA/SFA ratio was also decreased ($P<0.01$).

Table 5: Effects of dietary hesperidin supplementation on total weights of fatty acids (mg/100 g wet tissue) and fatty acid profile (%) in the *Longissimus lumborum* of rabbits (least square means)[1].

| | Hesperidin supplementation (g/kg feed) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | SEM | *P*-value |
| Total weights of fatty acids | 705 | 715 | 808 | 60.1 | 0.175 |
| C14:0 | 1.75[a] | 2.19[b] | 2.39[b] | 0.10 | <0.001 |
| C16:0 | 27.1 | 28.0 | 28.0 | 0.39 | 0.204 |
| C18:0 | 7.20[a] | 6.69[b] | 6.56[b] | 0.11 | <0.001 |
| C20:0 | 0.25 | 0.26 | 0.31 | 0.02 | 0.192 |
| C22:0 | 0.12 | 0.16 | 0.15 | 0.03 | 0.599 |
| Saturated fatty acids (SFA)[2] | 37.1 | 37.9 | 38.1 | 0.40 | 0.213 |
| C16:1n-7 | 2.79[a] | 3.73[b] | 3.64[b] | 0.24 | 0.016 |
| C18:1n-9 | 19.4 | 19.7 | 19.6 | 0.39 | 0.803 |
| Monounsaturated fatty acids (MUFA)[3] | 24.3 | 25.7 | 25.3 | 0.61 | 0.270 |
| C18:2n-6 | 20.4 | 19.3 | 19.8 | 0.27 | 0.085 |
| C18:3n-3 | 0.91 | 0.97 | 1.06 | 0.05 | 0.173 |
| C20:3n-3 | 0.63 | 0.59 | 0.54 | 0.03 | 0.173 |
| C20:4n-6 | 5.21[a] | 4.34[b] | 4.17[b] | 0.24 | 0.010 |
| C20:5n-3 | 0.09[a] | 0.08[a] | 0.04[b] | 0.01 | 0.010 |
| C22:5n-3 | 0.58[a] | 0.47[b] | 0.44[b] | 0.03 | 0.007 |
| C22:6n-3 | 0.08 | 0.13 | 0.05 | 0.03 | 0.172 |
| Polyunsaturated fatty acids (PUFA)[4] | 29.5[a] | 27.2[b] | 27.4[b] | 0.53 | 0.007 |
| PUFA series n-3 | 3.61 | 3.26 | 3.20 | 0.14 | 0.111 |
| PUFA series n-6 | 25.7[a] | 23.7[b] | 23.9[b] | 0.43 | 0.004 |
| n-6/ n-3 ratio | 7.36 | 7.45 | 7.74 | 0.42 | 0.803 |
| PUFA/SFA ratio | 0.80[a] | 0.72[b] | 0.72[b] | 0.02 | 0.009 |

SEM: standard error of the means.

[a,b] Means in a row with different superscripts are significantly different at $P<0.05$.

[1] 16 animals per experimental group.

[2] SFA: sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0 C20:0 and C22:0 (individual C12:0, C15:0, C17:0 and C19:0 are not presented).

[3] MUFA: sum of C14:1, C15:1, C16:1n-7, C17:1n-7, trans-C18:1 (total of trans-monoenoic isomers, mainly trans9-C18:1 and trans11-C18:1), C18:1n-9, C18:1n-7 and C20:1 (individual C14:1, C15:1, C17:1n-7, trans-C18:1, C18:1n-7 and C20:1 are not presented).

[4] PUFA: sum of C18:2n-6, C18:3n-3, C20:2, C20:3n-3, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3 and C22:6n-3 (individual C20:2 and C22:4n-6 are not presented).

Controversial results were obtained in previous studies. Dietary incorporation of α-tocopherol reduced the SFAs and increased the PUFAs (linoleic, eicosapentaenoic and docosahexaenoic) and MUFAs concentration in fresh rabbit meat (Castellini *et al.*, 1998; 2000). Rabbits fed diets containing oats, seeds rich in phenolic compounds, had a lower concentration of a-linolenic (C18:3n-3) and docosahexaenoic (C22:6n-3) and a higher concentration of oleic (C18:1n-9) compared to the control rabbits (Lopez-Bote *et al.*, 1998). Furthermore, dietary inclusion of chia seed, which contains a number of compounds with potent antioxidant activities: myricetin, quercetin, kaemperol and caffeic acid, caused a decrease in myristic (C14:0), palmitic (C16:0) oleic (C18:1), linoleic (C18:2n-6), a-linolenic (C18:3n-3) and arachidonic (C20:4n-6) concentration in the *LL* muscle of rabbits. However, no significant differences were found among the treatments for palmitoleic (C16:1) and stearic (C18:0) content in the previous study. Moreover, the SFAs, MUFAs and n-6/n-3 and saturated/unsaturated fatty acid ratios of the *LL* muscle decreased with increasing chia seed inclusion level (Peiretti and Meineri, 2008). At the same time, supplementation of rabbit diets with Spirulina (*Arthrospira platensis*), a rich source of phycocyanin and carotenoids, led to a significant increase in n-6 fatty acids; specifically C18:2n-6 and other polyunsaturated fatty acids levels (Peiretti and Meineri, 2011; Dal Bosco *et al.*, 2014).

These results demonstrate that hesperidin dietary supplementation did not affect meat and carcass quality characteristics or the extent of lipid oxidation in raw *LL* muscle stored at 4°C for up to 9 d. On the other hand, hesperidin incorporation in rabbit diet reduced the concentration of C20:4n-6, C20:5n-3 and C22:5n-3 fatty acids, and as a consequence, PUFAs and PUFA/SFA ratio were also decreased. Therefore, it may be concluded that further experimentation is needed to elucidate the action of hesperidin in rabbit metabolism and its effect on the quality characteristics of meat.

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