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Supplementation of *Rosemary* extract in the diet of Nero Siciliano pigs: evaluation of the antioxidant properties on meat quality

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In the present study, the effect of Rosmarinus officinalis *L. dietary supplementation on meat quality and oxidative stability of Nero Siciliano pigs was examined. During the growing–fattening period, 32 Nero Siciliano pigs were allotted into two treatment groups consisting of 8 replicates with 2 pigs per pen. For 90 days, the animals received a basal diet: one group (CTR) was not dietary supplemented, whereas the other group received (1 g/kg) rosemary extract (ROX). Supplementation with rosemary extract significantly improved the polyunsaturated fatty acid content of the meat, which showed higher values in the meat of the ROX group compared with the CTR group ($P < 0.001$); in detail, values for 18:2n-6 ($P = 0.001$), 20:4n-6 ($P = 0.001$), 22:6n-3 ($P = 0.012$) and PUFA/SFA ratio ($P = 0.004$) appeared to increase in the dietary supplemented group. On the other hand, atherogenic and thrombogenic indexes showed slight differences among the groups (IA: $P = 0.079$; IT: $P = 0.084$). Dietary supplementation with* R. officinalis *L. was not effective in protecting meat from oxidative deterioration, considering that the lipid oxidation, measured in meat across 5 days of refrigerated storage in darkness, progressed similarly in both the groups during storage ($P > 0.05$). Color measurement performed in the present study on meat samples from the two dietary treatments showed that redness decreased ($P = 0.046$) and hue values increased ($P = 0.036$), indicating that a deterioration of the initial color occurred and that the rosemary extract was ineffective in preventing color deterioration. Nevertheless, the lightness, yellowness and chroma color descriptors showed similar values in relation to dietary treatment ($P > 0.05$). Considering the nutritional value of meat as an important contributor to the overall quality, the results obtained in this study support the possibility of the dietary supplementation with* R. officinalis *L. extract in pigs as a functional additive in livestock feeding.*

Keywords: Nero Siciliano pig, rosemary extract, oxidative stability, meat

Implications

Rosmarinus officinalis L. extract-supplemented diet can influence the antioxidant properties of the meat of Nero Siciliano pigs, characterized by a high proportion of unsaturated fats and by a high susceptibility to lipid oxidation. Dietary supplementation with rosemary extract in pig diet induced an increase in the contents of the n-3 and n-6 polyunsaturated fatty acids in the *m. longissimus lumborum*, but it was not effective in improving the shelf-life of the meat; in fact, lipid oxidation and color deterioration of the meat progressed over storage duration. Rosemary extract could be used as a means of enhancing the nutritional properties of pork for the benefit of consumers.

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Introduction

Consumers are increasingly aware of the relationship between diet, health and well-being, resulting in choices of foods that are healthier and more nutritious compared with the past. Moreover, the appearance of fresh retail meat is a major determinant of its appeal to consumers, and, consequently, the sales of the product. Efficient protection of meat products against oxidative deterioration depends on the optimization of the parameters identified at critical points along the production chain. Research has extensively demonstrated that the diet of the animal can have a strong impact on the oxidative stability of meat, by modulating the oxidative status of the muscle (Aouadi *et al*., 2014). A high intake of oxidized lipids, such as the oxidized products of sensitive polyunsaturated fatty acids (PUFA) or of the pro-oxidants, and a low intake of nutrients involved in the

antioxidant defense system increase the meat oxidation processes. It is widely accepted that the oxidative deterioration of muscle components such as lipids and myoglobin is responsible for the deterioration of nutritional and sensory properties of meat and meat products (Faustman *et al.*, 2010).

In this context, the use of natural antioxidants in animal feed with the intention of replacing synthetic food preservatives, which have been found to cause long-term toxicological effects, including carcinogenicity, is gaining popularity as a strategy to improve the oxidative stability of meat (Nieto *et al.*, 2010). Many herbs and plant extracts, such as rosemary, thyme and oregano, have antimicrobial activities and antioxidant properties, which make them useful as natural animal feed additives (Faixovà and Faix, 2008). Several studies have been carried out to investigate the effects of dietary administration of natural antioxidants on the oxidative stability of meat and meat products. The greatest interest has been toward herbal products rich in phenolic compounds; one of which is *R. officinalis* L. (Labiatae), a common household plant grown in many parts of the world, which is recognized for its significant therapeutic benefits that include a potent antioxidant activity as well as anticancerogenic and antiviral properties (Aruoma *et al.*, 1992). Most constituents of rosemary are phenolic diterpenes, which may be considered as 'natural functional ingredients' (O'Connel and Fox, 2001). The major phenolic diterpene is carnosic acid (CA), a lipophilic antioxidant that prevents lipid peroxidation and disruption of biological membranes by scavenging singlet oxygen, hydroxyl radicals and lipid peroxyl radicals (Aruoma *et al.*, 1992). CA may give rise to carnosol (CAR) – another abietane diterpene – after enzymatic dehydrogenation, or to highly oxidized diterpenes such as rosmanol (Ros), epirosmanol and methoxyepirosmanol, often present in small concentrations in rosemary extracts but with a high antioxidant activities (Thorsen and Hildebrandt, 2003). CA has an antioxidant activity, approximately three times higher than that of CAR and seven times higher than that of the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole (Moñino *et al.*, 2008).

The use of rosemary as an additive is often limited due to its characteristic odor; therefore, commercial rosemary extracts are produced to extract components with high antioxidant activity while eliminating the essential oils that give rosemary its distinctive aroma. Puangsombat and Smith (2010) have demonstrated that the use of rosemary extract as an additive can effectively inhibit the formation of heterocyclic amines in cooked beef, suggesting that the addition of rosemary extracts is an important factor in decreasing carcinogenic compounds in cooked beef.

The exact fate and metabolism of compounds present in rosemary extract in the porcine monogastric digestive system is still unknown; nevertheless, considering the well-known benefits of the use of *R. officinalis* L. in animal feed, the aim of this study was to evaluate the influence of dietary rosemary extract on the oxidative stability of the meat of Nero Siciliano pigs, which is characterized by a high proportion of unsaturated fats and by a high susceptibility to lipid oxidation.

Material and methods

Animals and diets

All the procedures used in this research were in compliance with the European guidelines for the care and use of animals in research (Directive 2010/63/EU). The study was carried out on 32 Nero Siciliano pigs bred on an authorized farm; all pigs were in good general health. During the growing–fattening period, the animals, 16 castrated males and 16 females, were allotted into two dietary treatment groups on the basis of their live weight (33.5 ± 6 kg). All the pigs were housed in pens with two pigs per pen (one castrated male and one female). Pens, with grilled flooring made of metal, were located in the same room with controlled temperature and ventilation. In the front of the pen, a stainless steel trough was present. Water was supplied via nipples equipped with volume litter-counter at the back of the pen.

The animals were fed apelleted complete feed, rationed on the basis of 3% of the live weight supplemented either with 1 g/kg of a rosemary extract (ROX P® – Sevecom S.p.A.) (ROX group) or not (CTR group). ROXP® supplement (SEVECOM SpA., Italy; www.sevecom.it), a natural extract of *R. officinalis* L., is a hydrophobic powder, which is resistant to heating up to 110°C, with guaranteed minimum totals of 75 g/kg of antioxidants and of 16.5 g/kg of CA. ROXP® was integrated in the pelleted complete feed before the pelleting procedure, which was carried out at 70°C. The proportion of ingredients in the pelleted complete feed is reported in Table 1.

Pigs had free access to water. The trial lasted 90 days, coinciding with the growing–fattening period, preceded by a 15-day adaptation period to the experimental diet. The animals were slaughtered in an authorized commercial EU-licensed abattoir following the recommendations of European Union concerning animal care (Commission Regulation (EC) No 882/2004 of the European Parliament and Council). Every 35 days, individual BW of pigs was measured using an electronic balance. Feed intake of each pen was calculated for the entire experimental period by measuring the difference between the amount of feed administered and that remained in the trough. Based on the BW and daily feed intake, the average daily gain (ADG) and the feed conversion ratio (FCR = feed to gain ratio) of each piglet were calculated.

Collection and analysis of feed

Samples of the pelleted complete feed, obtained from the same batch for the whole trial, were collected for dry matter determination by oven-drying at 105°C for 24 h; for chemical analysis using the official methods of analysis (Association of Official Analytical Chemists (AOAC), 2007); and for the fatty acid profile determination using GC-FID (Table 1). In order to

Table 1 *Ingredients and composition of the pelleted complete feed*

Ingredients (g/kg of DM)	550
Corn	550
Broad bean	125
Peas bean	110
Sunflower meal (38% CP)	80
Wheat middling	70
Carob	30
Sugar cane molasses	13
Calcium carbonate	9
Sodium chloride	4
L-Lysine HCL	4
Dicalcium phosphate	4
Methionine–DL	1
Chemical composition (g/kg of DM)	
DM (%)	892
Moisture	108
CP (N × 6.25)	170
Crude Fat	38
Crude Fiber	60
Ash	41
NDF	183
ADF	74
ADL	20
Fatty acid classes (g/100 g FAME)[1]	
Saturated (SFA)	16.80
Monounsaturated (MUFA)	27.08
Polyunsaturated (PUFA)	56.12
n-3	3.68
n-6	52.44

DM = dry matter; FAME = fatty acid methyl esters.
Pelleted complete feed provided the following per kg: vitamin A (15 000 IU), vitamin D_3 (2000 IU), alpha-tocopherol (40 mg), vitamin B_1 (2 mg), vitamin B_2 (5 mg), vitamin B_6 (2 mg), vitamin B_{12} (0.02 mg), folic acid (1.75 mg), Zn (250 mg), Fe (300 mg), Mn (90 mg) and Cu (25 mg). Data were provided by the feed manufacturing industry: Mangimi Leone S.P.A., Italy.
[1]The concentration of fatty acid classes was expressed as g/100 g, considering 100 g the sum of the areas of all FAME identified.

quantify the principal phenolic compounds, feed samples of the pelleted complete feed integrated with ROXP® were collected, once a month, from Day 0 to Day 90 of the trial for a total of four samplings. Quantification of the principal phenolic antioxidants of the samples of the pelleted complete feed integrated with ROXP® was carried out by HPLC. Fifty grams of integrated CPF (Pelletted Complete Feed) was ground in a vertical hammer mill (Cyclotec 1093 Sample Mill) at 0.8 mesh; 50 mg of the ground sample was extracted with 25 ml of methanol: water (2 : 1, v/v) in an ultrasonic bath, avoiding over-heating. Each sample was centrifuged, filtered and analyzed by HPLC-RP. The system was controlled with HPLC EZ START 7.3 software. From the absorption spectra of sample solutions, absorbance at 230 nm was used. The column used was an Adsorbosphere HS C18-RP, 250 × 4.6 mm ID, 5 μm particle size (Alltech Italia Srl., Sedriano, Italy). For elution of the four compounds (Ros, rosmarinic acid (RA), CAR and CA), a binary acidified gradient was used consisting of solvents A (MeCN-H_2O-H_3PO_4; 65.1%:34.9%:0.02%) and B (MeCN-H_2O-H_3PO_4; 22%:78%:0.25%). The retention times were as follows: for Ros = 1.5 min, for RA = 3.9 min, for CAR = 7.4 min and for CA = 10.4 min. Quantification of phenolic antioxidants was carried out by an external standard method. Each phenolic compound was expressed as mg/kg of concentrate, and results show the mean value of the five individual samples of CPF integrated with ROXP®.

Measurement of pH, color and lipid oxidation of the meat samples

The pH_1 and pH_u values of *m. longissimus lumborum* were determined 45 min and 24 h *postmortem*, respectively, using a WTW 330/SET 1 (Weilheim, Germany) pH-meter, equipped with a Hamilton Double-pore glass piercing electrode and an automatic temperature compensator. At 24 h *postmortem*, after refrigerated storage at 4°C, samples of *m. longissimus lumborum* (L2-L5) were taken from the left half-carcass of each animal, were packaged in polystyrene trays covered with an oxygen permeable polyethylene film and were stored at 4°C. The color was measured on a 2.5-cm-thick slice of meat using the Commission Internationale de l'Eclairage/ International Commission on Illumination (1978) system color profile for lightness (L^*), redness (a^*) and yellowness (b^*) using a desktop photometer (Spectral scanner, DV Tecnologie d'Avanguardia, Padova, Italy) calibrated against a standard white tile using illuminant source D65. Before color evaluation, each sample was allowed to oxygenate at 4°C for 45 min covered with an oxygen permeable polyethylene film. After removing the polyethylene film, meat color was determined by taking one reading for the whole slice of each sample. The parameters referred to chroma (C^*) and hue (H^*) in raw pig *m. longissimus lumborum* were calculated according to the following formulas:

$$\text{Chroma} = \left(a^2 + b^2\right)^{1/2}$$

$$\text{Hue} = \tan^{-1}(b/a).$$

The thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Luciano *et al.* (2001). In brief, a 2.5 g portion of *m. longissimus lumborum* was finely minced using a knife and homogenized with 12.5 ml of distilled water. Trichloroacetic acid (12.5 ml; 10%, w/v) was added to precipitate proteins. Samples were filtrated and 4 ml of the filtrate was mixed with 1 ml of 0.06 M aqueous thiobarbituric acid. Samples were incubated in a water bath at 80°C for 90 min and the absorbance at 532 nm was measured. The assay was calibrated using standard solutions of 1,1,3,3,-tetra-ethoxypropane in trichloroacetic acid (5% w/v). Results were expressed as mg of malondialdehyde (MDA)/kg of muscle. Three replicates ($n = 3$) were run per sample. Color and TBARS were determined at 1, 3 and 5 days after storage at 4°C.

Lipid content and fatty acid composition of the meat

Lipid content in each sample was determined according to AOAC (2007). For the analysis of the acidic composition of intramuscular fats, in the individual lyophilized samples of *m. longissimus lumborum*, lipids were extracted using a

mixture of chloroform/methanol (2 : 1, v/v), and fatty acids methyl esters of the intramuscular fat were prepared by direct transesterification with sulfuric acid/methanol (1 : 9, v/v) of a weighed portion (15 mg) of the total lipids and analyzed using the high resolution gas chromatography technique. The fatty acid methyl esters (FAME) of the *m. longissimus lumborum* were analyzed by GC-FID (Agilent Technologies 6890 N, Palo Alto, CA, USA) with a split/splitless injector, a flame ionization detector and fused silica capillary column Omegawax 250, 30 m × 0.25 mm I.D., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). The column temperature was programmed: initial isotherm of 160°C (6 min), increment of 3°C/min and a final isotherm of 250°C (30 min). Temperature of the injector and detector was 250°C. Injection volume was 1.0 μl. The carrier gas used was helium (1 ml/min), and the split ratio was 1 : 50. Fatty acids were identified by comparing the relative retention times of FAME peaks from samples with standards from Supelco. Chromatogram peak areas were acquired and calculated using Chemstation software (Agilent). Concentration of each fatty acid was expressed as g/100 g, considering 100 g as the summation of the areas of all FAME identified. For each sample, the chromatographic analysis was repeated three times. On the basis of the identified fatty acids, the atherogenic index and the thrombogenic index were estimated using the equations proposed by Ulbricht and Southgate (1991).

Statistical analysis

For performance parameters, pen means served as the experimental unit for statistical analysis. Data were analyzed by ANOVA with the GLM procedure (v. 8.2, SAS Institute Inc., Cary, NC, USA; SAS/STAT, 2001), using treatment as the classification factor with the following model:

$$Y_{ijkl} = \mu + D_i + P_j + A_k(i) + \varepsilon_{ijkl}$$

where Y_{ijkl} are observations, μ the overall mean, D_i the fixed effect of diet i ($i = 2$), P_j the fixed effect of period j ($j = 5$ for BW; $j = 4$ for ADG and FCR), $A_k(i)$ is the random effect of animal k ($k = 32$) nested within diet i and ε_{ijkl} is the random residual.

Data of lipid (TBARS) and color stability parameters were analyzed using a mixed model (Proc. MIXED of SAS, Version 8.2, 2001), which included the effects of diet (CTR, ROX), time of storage (Time; days 1, 3 and 5) and their interaction as fixed factors and individual pig as a random effect. Data of the lipid content, pH values (pH_1 and pH_u) and fatty acid profiles were analyzed using a mixed model (Proc. MIXED of SAS, Version 8.2, 2001), which included the effect of the diet (CTR, ROX) as fixed factor and individual pig as a random effect. The effect of sex was initially included in the model but it was not significant ($P = 0.50$–0.90); thus, it was ultimately excluded. Least square means and root mean square error were calculated. Comparisons between LS means were performed using the Tukey test. Differences were considered significant at $P < 0.05$.

Results and discussion

Principal phenolic antioxidants of the ROXP® integrated in the complete pelleted feed

Several factors linked to plants, such as season and cultivation conditions, or to storage and extraction methods (i.e. ratio of water and ethanol in the solvent), can affect the composition and the biological activity of phenolic antioxidants present in the rosemary extract (Puangsombat and Smith, 2010). This is due to the differential potentials of antioxidant compounds with different polarity (Okoh *et al.*, 2010). Ros, RA, CAR and CA were identified and quantified in the complete pelleted feed integrated with rosemary extract after pelleting, because these phenolics are the major antioxidants present in rosemary. The sum of the identified phenolic compounds was 209 ± 5.0 mg/kg of concentrate integrated with ROXP®. In the rosemary extract, the phenolic compound with the highest content was RA (100 ± 3.0 mg/kg), followed by CAR (73 ± 2.5 mg/kg), CA (39 ± 1.0 mg/kg) and Ros (23 ± 0.8 mg/kg), a highly oxidized diterpene often present in small concentrations in rosemary extract, but with a high antioxidant activity (Thorsen and Hildebrandt, 2003). The contents of examined phenolic compounds of the four samples of concentrate integrated with ROXP® were unaffected by the storage time (90 days).

In vitam *performance traits*

The introduction of a new product (rosemary extract) to animal diet implies control of the growth performances of animals; thus, the *in vitam* performance traits of both the groups were measured during the trial. Final BW (ROX: 73.73 kg *v.* CTR: 69.48 kg; $P = 0.106$), average daily gain (ROX: 444 g/head per day *v.* CTR: 410 g/head per day; $P = 0.285$), daily feed intake (ROX: 1.582 kg per day *v.* CTR: 1.642 kg per day; $P = 0.331$) and feed conversion rate (ROX: 4.06 kg/kg *v.* CTR: 4.36 kg/kg; $P = 0.417$) showed similar values in both the groups (data not shown). Mean values of water intake showed no significant differences between the two groups (ROX: 3.58 l per day *v.* CTR: 3.70 l per day; $P = 0.524$).

The inclusion of rosemary had no beneficial effects on growth performances, according to the observations of Cullen *et al.* (2005). No previous research has been found in the literature regarding the effects of rosemary on nutrient digestibility. Nevertheless, although the inclusion of rosemary extract had no beneficial effects on growth performance, several studies have shown its effects on fatty acid composition and lipid oxidation.

Lipid oxidation and color stability

The lipid content of the *m. longissimus lumborum* was unaffected by the dietary treatment (ROX: 3.00 g/100 g *v.* CTR: 3.12 g/100 g; $P = 0.629$), as well as pH_1 (ROX 6.28 *v.* CTR 6.19; $P = 0.666$) and pH_u (ROX 5.53 *v.* CTR 5.55; $P = 0.842$) (data not shown).

Table 2 reports the effect of the dietary treatment and storage time on lipid oxidation and color stability in meat

Table 2 *Effect of rosemary extract supplementation and storage time on the lipid oxidation (TBARS)[1] and color stability in porcine* m. longissimus lumborum *stored over 5 days at 4°C*

	Dietary treatment (D)[2]									
	CTR			ROX				*P*-value		
Storage time (T)	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	r.m.s.e.	T	D	T × D
TBARS	0.22[b]	0.054[a]	0.039[ab]	0.021[b]	0.046[a]	0.040[ab]	0.014	<0.010	0.344	0.400
Lightness (*L**)	60.90	61.64	62.21	61.24	64.31	63.81	3.28	<0.001	0.223	0.582
Redness (*a**)	14.44	15.42	13.69	13.41	13.62	12.91	1.53	<0.001	0.046	0.455
Yellowness (*b**)	15.09	15.60	15.54	15.57	15.51	16.63	1.71	<0.001	0.208	0.836
Chroma (*C*)	20.95	22.00	20.74	20.64	21.46	21.14	1.46	<0.001	0.790	0.577
Hue (*H*)	0.81	0.79	0.85	0.86	0.89	0.91	0.84	<0.001	0.036	0.707

[a,b]Values within a row with different superscripts significantly differ at $P < 0.05$.
[1]TBARS was expressed as mg of malonaldehyde/kg of muscle.
[2]Dietary treatments: CTR, basal pelleted complete feed; ROX, basal pelleted complete feed supplemented with 1 g/kg of a rosemary extract (ROX P® – Sevecom S.p.A.).

stored over 5 days at 4°C. Lipid oxidation, expressed as TBARS (mg malondialdehyde/kg meat), was significantly affected by the time of storage ($P < 0.010$); in particular, TBARS significantly ($P < 0.05$) increased for all treatments at the beginning of the storage (Day 1 to Day 3) and then began to decrease (Day 3 to Day 5). According to Melton (1983), although malonaldehyde is a secondary product of lipid oxidation, it does not necessarily mean that the TBARS value continues to increase throughout storage. The low TBARS values could be the results of malonaldehyde reactions with proteins (Nassu *et al.*, 2003). Specifically, TBARS value fluctuation during the storage period could be due to the production of hydrogen peroxide, during the oxidation of oxymyoglobin to metmyoglobin, the presence of which determines the instability of malondialdehyde (Kostka and Kwan, 1989). Nevertheless, neither the diet ($P = 0.344$) nor the dietary treatment × storage time interaction ($P = 0.400$) affected lipid oxidation, measured in meat across 5 days of refrigerated storage in darkness, indicating a lack of effect of the rosemary extract used on the observed trend of development in lipid oxidation. Results are in accordance with Aouadi *et al.* (2014) in lambs receiving oral administration of *R. officinalis* essential oil and with Nieto *et al.* (2010) in ewes fed a diet supplemented with distilled rosemary leaf. This could be due to the concentration of the extract added, which was not sufficient to improve the shelf life of the meat. Probably, a significant loss of phenolic compounds occurred as lipid oxidation progressed, a finding of which may be understood on the basis of antioxidative activity and concomitant degradation of the phenolic compounds due to the development of free radicals in the primary stage of oxidation (radical trapping stage) during the chain-breaking antioxidant process (Masuda *et al.*, 2001).

Although many factors can influence the stability of meat color, the oxidation of myoglobin by free radicals or by lipid peroxidation products is predominant. The stability of meat color over time is determined by the extent of myoglobin oxidation and the accumulation of metmyoglobin, causing the loss of the appealing color of fresh meat, which can be instrumentally monitored by measuring the color descriptors (Mancini and Hunt, 2005). In particular, the decrease in redness (*a** values) and the increase in hue values (*H**) appear to be suitable descriptors of meat color deterioration occurring over time; these descriptors impart a less-intense red color and a worse appearance of the meat (Nieto *et al.*, 2010). The variation of these descriptors and of hue values in particular have been proved to be highly associated with the accumulation of metmyoglobin on meat surface (Luciano *et al.*, 2011), as a consequence of the increased oxidation of oxymyoglobin to metmyoglobin (Chan *et al.*, 1997). The meat samples of the two dietary treatments (Table 2) showed that redness decreased ($P = 0.046$) and hue values increased ($P = 0.036$). Moreover, in all the samples, lightness (Table 2) increased with storage time ($P < 0.001$). It has been reported than an increase in lightness value may be related to an increase in metmyoglobin formation (Nieto *et al.*, 2010). Therefore, in the present study, it could be hypothesized that the high extent at which lipid oxidation developed with storage time might have contributed to promote color deterioration and that the dietary rosemary extract did not exert effects on color stability, as also observed by Aouadi *et al.* (2014). All the color descriptors showed similar values for meat in relation to dietary treatment × storage time interaction.

The level of bioavailability of phenolic compounds typically found in *R. officinalis* extracts is still controversial, and it has been reported that some compounds typically found in rosemary extract are bioavailable in animals, including humans. The bioavailability seems to be due to the transfer of phenolic compounds from feed to animal (Faixovà and Faix, 2008). An *in vitro* fermentation study indicated that rosemary compounds were not fermented in simulated rumen conditions indicating their 'potential availability' for absorption in the bovine intestine (O'Grady *et al.*, 2006). The oral administration of CA from *R. officinalis* in a single dose to rats showed the absorption of the phenolic compound into the bloodstream and the recovery in feces, which represent its main elimination route (Doolaege *et al.*, 2011).

Several evidences suggest that lipid and color stability in meat are linked (Aouadi *et al.*, 2014), and although the exact mechanisms of such connection are not yet fully understood, it has been reported that strategies able to reduce the extent of lipid oxidation often reduce myoglobin oxidation with a consequent improvement in meat color stability (Faustman *et al.*, 2010). In the present study, the rosemary extract was not effective in protecting meat from oxidative deterioration, but, at the same time, the dietary rosemary extract increased the concentration of oxidizable fatty acids in muscle. In fact, as reported by Gray *et al.* (1996), although many factors can influence the stability of meat color, the oxidation of myoglobin by free radicals or by lipid peroxidation products is predominant. Moreover, Mourot (2009) reports that the susceptibility of meat to oxidation depends on factors such as muscle fatty acid composition; specifically, the susceptibility to oxidation increases with increasing unsaturation. From this point of view, it can be concluded that the incorporation of rosemary extract at 1 g/kg of pig diet was not sufficient to improve the meat antioxidant status.

Fatty acid profile

Dietary administration of rosemary extract influenced the fatty acid composition of meat. The most significant differences among the fatty acids of nutritional interest of the *m. longissimus lumborum* were observed for the 16 : 0 ($P < 0.05$) among the saturated fatty acids, for the 18:1n-9 ($P < 0.001$) among the monounsaturated fatty acids and for the 18:2n-6 ($P = 0.001$), 20:4n-6 ($P = 0.001$) and 22:6n-3 ($P = 0.012$) among the PUFAs (Table 3).

Table 3 *Effect of rosemary extract supplementation on the fatty acid profile (g/100 g FAME)*[1]*, ratios and quality indexes of intramuscular fat in porcine* m. longissimus lumborum

	Dietary treatment (D)[2]			
	CTR	ROX	r.m.s.e.	*P*-value
Saturated fatty acids (SFA)	41.57	41.26	1.95	0.755
14:0	1.08	0.87	0.15	0.011
15:0	0.03	0.05	0.02	0.102
16:0	24.03	22.56	1.66	0.035
iso-16:0	0.58	1.25	0.41	0.004
17:0	0.27	0.27	0.06	0.965
iso-18:0	0.52	0.91	0.37	0.039
18:0	14.03	14.78	1.29	0.404
20:0	0.24	0.29	0.04	0.028
22:0	0.12	0.25	0.07	<0.001
23:0	0.07	0.06	0.06	0.558
Monounsaturated fatty acids (MUFA)	49.50	44.70	1.78	<0.001
16:1	3.16	2.98	0.45	0.407
17:1	0.27	0.26	0.05	0.647
18:1n-9	40.50	36.24	1.35	<0.001
18:1n-7	4.46	4.44	0.47	0.923
20:1n-9	1.11	0.78	0.24	<0.001
n-3 Polyunsaturated fatty acids (PUFA)	0.80	1.39	0.42	0.043
18:3n-3	0.22	0.25	0.05	0.136
20:3n-3	0.06	0.05	0.03	0.762
20:5n-3	0.07	0.11	0.04	0.057
22:5n-3	0.31	0.46	0.28	0.247
22:6n-3	0.14	0.27	0.08	0.012
n-6 Polyunsaturated fatty acids (PUFA)	8.13	12.65	2.35	<0.001
18:2n-6	6.21	9.09	1.54	0.001
20:2n-6	0.25	0.32	0.09	0.131
20:4n-6	1.38	2.70	0.71	0.001
22:4n-6	0.20	0.44	0.10	<0.001
22:5n-6	0.09	0.10	0.07	0.830
Unsaturated/saturated (UFA/SFA)	1.41	1.42	0.11	0.684
Polyunsaturated/saturated (PUFA/SFA)	0.21	0.34	0.08	0.004
n-3/n-6	0.10	0.11	0.07	0.545
Atherogenic index	0.49	0.44	0.05	0.079
Thrombogenic index	1.11	0.99	0.14	0.084

[1]The concentration of fatty acid classes was expressed as g/100 g, considering 100 g the sum of the areas of all fatty acid methyl esters (FAME) identified.
[2]CTR, basal pelleted complete feed; ROX, basal pelleted complete feed supplemented with 1 g/kg of a rosemary extract (ROX® – Sevecom S.p.A.).

With regard to the fatty acid classes, ratios and quality indexes of intramuscular fat of the *m. longissimus lumborum* (Table 3), the most significant differences were observed for the total PUFAs ($P = 0.001$), for the PUFAs of the n-6 ($P < 0.001$) and n-3 series ($P < 0.05$) and for the PUFA/SFA ratio ($P = 0.004$). The atherogenic and thrombogenic indexes, which characterize the atherogenic and thrombogenic potential of a vegetable or animal food (Fehily *et al.*, 1994), showed no significant differences between the two groups (IA: $P = 0.079$; IT: $P = 0.084$), although slightly lower values were observed for the quality indexes in the ROX group than in the CTR group.

The significant increase in the content of the PUFAs in the meat of the ROX group could be due to powerful antioxidant activity of phenolic compounds against the oxidative deterioration of these food components. A primary mechanism for a phenolic antioxidant includes the trapping and stabilizing of species generated from the radical chain oxidation of food components. Although the first stage (radical trapping stage) is a reversible process, the second stage (radical termination stage) is irreversible and must produce stable radical termination compounds (Masuda *et al.*, 2001), thus terminating the chain reaction and preserving PUFAs by the synthesis of hydroperoxides (Chen *et al.*, 1992). Another explanation could be that the rosemary extract increases the activity of the delta-5 desaturase (Δ5d) and delta-6 desaturase (Δ6d), which are the enzymes involved in the synthesis of 20:5n-3 and 22:6n-3 derived from the 18:3n-3 (Mourot, 2009). This could explain the fact that dietary rosemary extract by increasing the concentration of oxidizable fatty acids in muscle partially cancelled its antioxidant capacity on the muscle and it did not have any impact on the shelf-life of the meat, as also observed by Aouadi *et al.* (2014). Li and Liu (2012) point out that there are many examples where color and lipid stability appear to be linked and, in particular, the lipid oxidation is a catalyst for the discoloration.

From a nutritional point of view, the preservation of the PUFAs by the peroxidation means an improvement in the nutritional quality of the meat. In fact, the beneficial effects of the longer chain n-3, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) in reducing the risk for cardiovascular disease, cancer and type-2 diabetes, as well as their critical roles for proper brain function, visual development in the fetus and for maintenance of neural and visual tissues throughout life, are well-recognized (Barceló-Coblijn and Murphy, 2009). A recent meta-analysis of epidemiological studies has casted doubt on the relationship between saturated fatty acids and cardiovascular diseases (Siri-Tarino *et al.*, 2010). It is not completely clear which should be the optimal n-3/n-6 value for the human nutrition; a strong imbalance toward high dietary intakes of n-6 PUFA at the expenses of n-3 is positively correlated with a number of widespread human diseases. The studies on the relationship between n-3/n-6 ratio and the pathogenesis of many diseases indicate that the optimal ratio may vary with the disease or the condition under consideration; this is consistent with the fact that many diseases are multigenic and multifactorial. In general, a higher ratio of n-3/n-6 is more desirable in reducing the risk for several chronic diseases; several international organizations have recommended a dietary n-3/n-6 ratio between 1:4 and 1:10 (McDaniel *et al.*, 2013). In our study the n-3/n-6 ratio was within this range.

Conclusions

The dietary supplementation with *R. officinalis* L. for 90 days in Nero Siciliano pigs induced an improvement in meat fatty acid profile; in particular, the content of polyunsaturated fatty acids of the n-3 and n-6 series in the *m. longissimus lumborum* was increased. On the other hand, rosemary extract dietary supplementation was not effective in improving the shelf-life of the meat; in fact, lipid oxidation and color deterioration in meat progressed over storage duration. In this direction, it could be of interest to further examine the effect of dietary rosemary extracts on meat oxidative stability using different storage and display conditions of meat, such as modified atmosphere packaging. In conclusion, considering the nutritional value of meat as an important contributor to the overall quality, the results obtained in this study support the possibility of the dietary supplementation with *R. officinalis* L. extract in pigs as a valid strategy to ensure quality of the production.

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Conflicts of Interest

The authors do not have any conflict of interest to declare.

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