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Polyphenolic Transmission to Segureño Lamb Meat from Ewes' Diet Supplemented with the Distillate from Rosemary (*Rosmarinus officinalis*) Leaves

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The aim of the present work is to study whether the introduction of rosemary plant byproduct, from plant steam distillation, in daily Segureña sheep feeding allows the transfer of active antioxidant components to lamb meat, without detriment to the animal productivity. For this, 36 Segureña ewes were assigned randomly to three homogeneous groups. One group was fed a basal diet as a control and the diet of the other two groups was modified by substituting 10 or 20% of the control diet (respectively) with distilled rosemary leaves. Chromatographic analysis allowed the identification of 11 polyphenolic components previously identified in the rosemary and basal diet pellets, respectively. Among them, rosmarinic acid, carnosol, and carnosic acid were the phenolic components that had a significantly increased presence ($P < 0.05$) in the lamb meat from sheep mothers fed this aromatic herb, when compared to the control group. The incorporation of this byproduct into the animal diet favored the antioxidant capacity of these lamb meat samples. Fresh meat produced on rosemary had higher total ferric reducing antioxidant power (FRAP) ($P < 0.05$), greater ability to reduce $ABTS^{\bullet+}$, and lower IC_{50} ($DPPH^{\bullet}$) ($P < 0.05$) values when compared to the control group. Because no statistically significant differences were detected among the results obtained from the lamb meat belonging to the different ewe groups fed rosemary leaf extract (10 or 20%), it can be concluded that the incorporation of distilled rosemary leaves at a rate of 10% of the ewes' diet should be enough to improve the lamb meat antioxidant status.

KEYWORDS: Segureño lamb meat; *Rosmarinus officinalis*; polyphenolic transmission; antioxidant status

INTRODUCTION

Ruminant animals produce a high proportion of the quality dietary protein consumed by human populations throughout the world. In the European Union, approximately 4% of the meat consumed is of ruminant origin (*1*), Spain being the second-greatest producer of lamb meat with 239,500 t pear year (*2*).

The value of ruminant animals lies in their ability to utilize low-quality feeds, upgrading low-quality inputs to high-quality outputs. Plant secondary metabolites have, in the past, been considered generally as a source of antinutritional factors, and not as a source of exploitable, performance-enhancing compounds. Recent and continuing changes to legislation controlling the use of animal feed additives have stimulated interest in bioactive secondary metabolites as alternative performance enhancers (*3*). They are broadly compatible with current thinking on the future of agriculture and food in Europe and with consumer opinion.

Some of the aromatic plants that are widespread in the Mediterranean area have already been studied for their antioxidant activity, including sage, rosemary, and thyme (*4*−*9*). The search for natural antioxidants in the wastes of aromatic plants has become an alternative to synthetic antioxidants in the food and pharmaceutical industries (*5, 9*). These authors considered the study of the material remaining after distillation potentially interesting, as a result of the water-soluble properties of phenolic compounds that rarely form part of essential oils. Phenolic compounds tend to be water-soluble because they frequently occur combined as glycosides, and they are usually located in the cell vacuole (*10*).

Rosemary (*Rosmarinus officinalis* L.) extract contains antioxidant compounds, the most active being phenolic diterpenes such as carnosol, carnosic acid, rosmanol, epirsomanol, isorosmanol, and methyl carnosate, and other phenolic acids, such as rosmarinic acid (*11*). Carnosic acid is the major phenolic constituent present in rosemary leaves with an antioxidant activity approximately 3 times higher than that of carnosol and 7 times higher than that of the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole (*12*).

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The beneficial antioxidant effects of rosemary extract or oleoresin, as a result of direct addition, have been studied extensively and reported in a variety of meat types, including beef (*13, 14*) and beef products (*15*), pork (*16*) and pork products (*17*), turkey (*18*), and goat-meat products (*19*). The scientific literature contains limited information regarding supplementation of animal diets with rosemary leaves.

Reduced levels of lipid oxidation in broiler meat have been reported as a result of rosemary oleoresin supplementation (*20*), but supplementation of hen diets with rosemary extract did not significantly improve the oxidative stability of the eggs produced (*21*); however, Botsoglou et al. (*22*) reported that dietary supplementation with rosemary leaves delayed lipid oxidation of eggs.

O'Grady et al. (*23*) published an assessment of the effect of dietary supplementation with tea catechins and rosemary extract (RE) on the quality of fresh beef. For these authors, this dietary supplementation of 1000 mg of RE/animal/day did not affect plasma total antioxidant status or longissimus dorsi α-tocopherol concentrations, pH, color, lipid stability, or sensory properties. Also, Goni et al. (*24*) concluded that the administration of grape pomace and vitamin E in chicken diets did not affect either the growth performance or the nutrient digestibility, but did increase meat antioxidant activity.

When all of these considerations are taken into account, the supplementation of ewe diets with natural antioxidant compounds, as alternative to synthetic antioxidant additives and as a means of improving lamb-meat quality, merits investigation. To our knowledge, pertinent research into the use of distilled aromatic plants in ewe feeding has never been accomplished.

The aim of the current study was to investigate whether the application of dietary rosemary leaves (from the distillate) to sheep mothers affects the lamb-meat polyphenolic composition and its antioxidant capacity.

MATERIALS AND METHODS

Reagents. All of the chemicals used were of AnalaR grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,4,6-tripyridyl-*s*-triazine (TPTZ), $FeSO_4 \cdot 7H_2O$, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and potassium persulfate ($K_2S_2O_8$) were purchased from Sigma-Aldrich (Madrid, Spain). CH_3COOH, CH_3COONa, HCl, and $FeCl_3 \cdot 6H_2O$ were obtained from Scharlau Chemie S.A. (Barcelona, Spain). Methanol, ethanol, acetonitrile, and water (HPLC grade) were bought from J. T. Baker (Mallinckrodt Baker B.V., Deventer, The Netherlands). Petroleum ether and formic acid, analytical grade, were purchased from Scharlau Chemie S.A. Authentic standards of gallic acid, caffeic acid, ferulic acid, coumaric acid, naringin, hesperidin, luteolin, rosmarinic acid, apigenin, genkwanin, carnosol, and carnosic acid were obtained from Sigma-Aldrich (Madrid, Spain).

Animals and Diets. Thirty-six Segureña ewe mothers were assigned randomly, on the bases of their age and body condition, to three homogeneous groups. The average values of these parameters were 3.2 years old and 2.63 (±0.15), respectively. Body condition was calculated according to the method described by Russel et al. (*25*).

Sheep in the control group were given a basal diet (BD) consisting of 1.3 kg/animal/day. The diets of the other two groups were modified by substituting 10 or 20% of the BD (respectively) with distilled rosemary leaves, using pellets consisting of 50% barley and 50% distilled rosemary leaves. The basal diet (**Table 1**) was provided by Cargill Animal Nutrition (Torre Pacheco, Murcia, Spain). Steam-distilled rosemary leaves were obtained from a local grower. Leaves were steam-distilled for 3 h using a distillation system with a stainless steel steam boiler.

Animals on these diets were fed for 240 days, coinciding with the gestation and lactation periods in order to study polyphenolic transmission to the lamb meat. After the lactation period, when the lambs reached a weight of 13 (±1) kg, animals (from the three groups assayed) were fed with commercial fattening pellets until they reached the slaughter weight of 25 (±2) kg.

Table 1. Chemical Composition of the Concentrated Basal Diet

nutrient	concentrate[a] (g/kg of dry matter)
ash	76.0
crude protein (CP)	175.5
fat	30.9
neutral detergent fiber (NDF)	366.4
digestible NDF (%)	25.0
acid detergent fiber	190.8
nonproteic nitrogen	0.5
ruminal degradable protein (RDP), % of CP	64.7
nonfiber carbohydrate	267.9
adjusted total starch	90.7
net energy lactation (mcal/kg)	1.5
total soluble RDP	43.3
ruminal undegradable protein	55.6
calcium	10
phosphorus	5.7
vitamin A (IU/g)	14.9
vitamin D (IU/g)	3.1
vitamin E (IU/g)	32.4
magnesium	3.4
selenium (mg/kg)	0.72
zinc (mg/kg)	154.4
total methionine	2.6
total lysine	6.7

[a] Formulated using the following ingredients/tonne: wheat bran, 266.5 kg; scale soy, 250 kg; barley, 150 kg; malt comb, 80 kg; sunflower oil (30%), 70 kg; rye, 70%; honey bean, 41.6 kg; corn flour, 36.7 kg; calcium carbonate, 14.9 kg; molasses-cane, 10 kg; vitaminic and mineral feed additives, 10.4 kg. Data provided by Cargill Animal Nutrition (Torre Pacheco, Murcia, Spain).

The sheep and lambs were reared at the CIFEA Research Center (Consejería de Agricultura, Región de Murcia, Spain). Weekly weighing of the animals was taken until the lambs reached their slaughter weight. Animals were slaughtered according to the technical regulations of the Spanish Health Department (RD 147/1993).

Meat Sampling. Fresh samples (24 h postslaughter) of meat from the front legs (M. deltoideus) and the abdominal wall (M. obliquus externus abdominis) were cut into pieces, vacuum-packaged, and stored at −80 °C until analysis. The selection of these muscles was made according to consumer preferences, because they are known to be the most flavorful meat of the lambs.

Polyphenol Extraction. Basal diet grains and rosemary distillate pellets were dried in a forced-air dryer at 35 °C for 48 h (until they reached a constant weight) and then ground to pass a 2 mm sieve.

Chopped-meat samples were lyophilized (VirTis, 6K BTEL-85 freeze drier, Ucoa-erloss, Madrid, Spain) and kept in a dry atmosphere until analysis.

Dried samples (0.5 and 1.5 g of plant material and meat, respectively) were extracted first with 20 mL of petroleum ether, with stirring, and taken to dryness at room temperature. Second, they were extracted using 150 mL of methanol in a Soxhlet extractor (B-811) (Buchi, Flawil, Switzerland) for 2 h under a nitrogen atmosphere. Methanolic extracts were taken to dryness at 40 °C, under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Buchi). The residue was redissolved in methanol and made up to 5 mL. The extracts were kept in vials at −80 °C until their corresponding analysis.

The polyphenolic qualitative and quantitative compositions of the three diets assayed are described in **Table 2**.

DPPH$^{\bullet}$ Radical-Scavenging Activity. The scavenging activities of the meat methanolic extracts were measured according to the method described by Brand-Williams et al. (*26*). Methanolic extracts (500 μL) at different concentrations (400 μL/mL to 25 μL/mL) were added to 1 mL of methanolic DPPH$^{\bullet}$ solution (0.1 mM). The estimated time of reaction (20 min) was determined by considering the reduction of the absorbance at 517 nm (monitored every 5 min) until the reaction curve reached a plateau. The absorbance was measured at room temperature, in darkness, against a blank (500 μL of sample plus 1 mL of methanol).

Table 2. Dietary Administration of Polyphenols Present in the Concentrate-Based Diet and in the Rosemary Pellet (50% Barley−50% Distilled *Rosmarinus officinalis* L. Leaves)

polyphenol	control diet[a] (mg/kg)	control diet−10% rosemary leaves[a] (mg/kg)	control diet−20% rosemary leaves[a] (mg/kg)
gallic acid	178.88 ± 29.672	163.61 ± 26.359	148.34 ± 23.045
caffeic acid	71.21 ± 12.333	78.17 ± 15.723	85.14 ± 19.114
ferulic acid	32.68 ± 2.568	41.87 ± 4.364	51.05 ± 6.169
coumaric acid	18.69 ± 0.844	33.62 ± 1.456	48.54 ± 2.068
naringin	46.29 ± 8.701	51.05 ± 9.242	55.82 ± 9.784
hesperidin	111.97 ± 17.819	284.42 ± 30.003	456.88 ± 42.196
luteolin	12.45 ± 1.255	17.49 ± 2.213	22.54 ± 3.171
rosmarinic acid	7.02 ± 0.326	140.44 ± 4.950	273.86 ± 9.583
apigenin	9.27 ± 0.631	12.99 ± 1.234	16.72 ± 1.837
genkwanin	120.06 ± 20.972	755.94 ± 119.787	1391.81 ± 218.601
carnosol	72.26 ± 3.944	374.55 ± 29.063	676.83 ± 62.070
carnosic acid	68.22 ± 1.532	107.71 ± 8.077	147.20 ± 14.621

[a] Data are the mean of three independent replicates from 4four pellet fabrications ($n = 12$) ± standard deviation.

Table 3. Lamb Meat (M. deltoideus) Polyphenolic Profile[a]

	M. deltoideus		
polyphenol	control (mg/kg of fresh meat)	10% (mg/kg of fresh meat)	20% (mg/kg of fresh meat)
caffeic acid	1.47 ± 0.03 a	1.41 ± 0.053 a	1.41 ± 0.047 a
ferulic acid	0.77 ± 0.007 a	0.79 ± 0.059 a	0.55 ± 0.030 a
coumaric acid	0.85 ± 0.006 a	0.86 ± 0.031 a	0.86 ± 0.008 a
naringine	2.42 ± 0.038 a	2.42 ± 0.027 a	2.43 ± 0.041 a
hesperidin	2.54 ± 0.030 a	2.64 ± 0.097 b	2.58 ± 0.036 ab
luteolin	0.36 ± 0.110 b	0.27 ± 0.025 a	0.29 ± 0.046 ab
rosmarinic acid	0.00 ± 0.000 a	1.17 ± 0.007 b	1.09 ± 0.220 b
apigenin	0.08 ± 0.005 a	0.08 ± 0.005 a	0.07 ± 0.022 a
genkwanin	0.03 ± 0.029 a	0.05 ± 0.074 a	0.02 ± 0.009 a
carnosol	2.41 ± 0.526 b	5.17 ± 1.823 a	4.77 ± 0.841 a
carnosic acid	7.09 ± 0.531 c	23.85 ± 4.385 a	18.46 ± 2.612 b

[a] Different letters denote significant differences among treatments ($P < 0.05$) ± standard deviation.

The absorbance of the control (500 μL of methanol in 1 mL of DPPH$^{\bullet}$ solution) was measured daily. All of the assays were conducted in triplicate.

The percentage activity for the DPPH$^{\bullet}$ technique was calculated according to

$$\% \text{ decoloration} = [1 - (\text{Abs sample}/\text{Abs control})] \times 100 \quad (1)$$

The results were expressed as the inhibitory concentration of the fresh meat necessary to decrease the DPPH$^{\bullet}$ absorbance by 50% (IC_{50}). Concentrations are expressed in milligrams per milliliter.

ABTS$^{\bullet+}$ Radical Cation Decoloration Assay. The Trolox equivalent antioxidant capacity (TEAC) assay was used for the determination of the meat antioxidant activity, according to the method described by Re et al. (*27*). The ABTS radical cation, ABTS$^{\bullet+}$, was produced by reacting 14 mM ABTS with an equal volume of 4.9 mM potassium persulfate (final concentration = 7 mM ABTS in 2.45 mM potassium persulfate). The mixture was incubated in the dark at room temperature, 12−16 h before use. The ABTS$^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. Meat polyphenolic extracts (10 μL) or Trolox standard (0−20 μM in ethanol) were added to 1.0 mL of the diluted ABTS$^{\bullet+}$ solution, and the absorbance was read at 30 °C, 1−6 min after the initial mixing. All of the absorbance readings were taken against a blank containing 10 μL of methanol plus 1 mL of ABTS$^{\bullet+}$ solution. Results are expressed as micromolar Trolox equivalent per gram of fresh meat.

Ferric Reducing Ability Assay (FRAP). The ferric reducing ability of polyphenolic meat extracts was measured according to a modified method developed by Descalzo et al. (*28*). Antioxidant compounds are able to reduce ferric to ferrous TPTZ, which develops a blue color with an absorption maximum at 593 nm. To prepare the FRAP reagent, a mixture of 0.1 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride (10:1:1) was made. To 1.2 mL of reagent was added 0.04 mL of methanolic extract and 0.12 mL of deonized water. Readings at the maximum absorption (593 nm) were taken every 15 s, and the reaction was monitored for up to 60 min. Endogenous meat Fe^{II} content ($FRAP_o$) was determined with a TPTZ/HCl solution without the presence of ferric chloride in the reaction mixture.

Table 4. Lamb Meat (M. obliquus externus abdominis) Polyphenolic Profile[a]

	M. obliquus externus abdominis		
polyphenol	control (mg/kg of fresh meat)	10% (mg/kg of fresh meat)	20% (mg/kg of fresh meat)
caffeic acid	1.45 ± 0.089 a	1.38 ± 0.057 a	1.39 ± 0.058 a
ferulic acid	0.77 ± 0.003 a	0.77 ± 0.004 a	0.64 ± 0.031 a
coumaric acid	0.86 ± 0.004 a	0.86 ± 0.009 a	0.85 ± 0.005 a
naringin	2.42 ± 0.047 a	2.42 ± 0.022 a	2.41 ± 0.014 a
hesperidin	2.58 ± 0.094 a	2.63 ± 0.067 a	2.56 ± 0.025 a
luteolin	0.32 ± 0.090 a	0.30 ± 0.111 a	0.31 ± 0.074 a
rosmarinic acid	0.00 ± 0.000 b	1.17 ± 0.004 a	1.17 ± 0.003 a
apigenin	0.08 ± 0.007 a	0.08 ± 0.002 a	0.08 ± 0.005 a
genkwanin	0.02 ± 0.005 a	0.02 ± 0.018 a	0.02 ± 0.005 a
carnosol	2.44 ± 0.358 b	3.85 ± 1.376 a	5.09 ± 1.140 a
carnosic acid	7.19 ± 0.453 b	21.21 ± 3.908 a	19.32 ± 2.932 a

[a] Different letters denote significant differences among treatments ($P < 0.05$) ± standard deviation.

Table 5. Antioxidant Capacity of the Meat Tissues Studied[a]

	Fe^{2+} (mM)/g	
	M. deltoideus	M. obliquus externus abdominis
control	0.55 ± 0.164 b	0.48 ± 0.043 b
10%	0.94 ± 0.147 a	0.82 ± 0.271 a
20%	1.13 ± 0.208 a	0.89 ± 0.202 a

[a] Different letters denote significant differences among treatments ($P < 0.05$) ± standard deviation.

Ferrous sulfate solutions ($FeSO_4 \cdot 7H_2O$) of 0−2 mM were used to obtain the calibration curve, and results were expressed as Fe^{II} equivalent per gram of fresh meat.

HPLC Analysis of Polyphenols. For the HPLC analysis, a method adapted from Zheng and Wang (*29*) was performed on a reverse-phase Zorbax SB-C18 column (4.6 × 250 mm, 5 μm pore size, Hewlett-Packard) using a guard column (Zorbax SB-C18 4.6 × 125 mm, 5 μm pore size, Hewlett-Packard) at ambient temperature. Extracts were passed through a 0.45 μm filter (Millipore SAS, Molsheim, France), and 20 μL was injected in a Hewlett-Packard system equipped with a G1311A quaternary pump and a G1315A photodiode array UV−vis detector. The mobile phase was acetonitrile (A) and acidified water containing 5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A; followed by a 10 min hold before return to the initial conditions. The flow rate was 1.0 mL/min, and the wavelengths of detection were set at 280 and 330 nm. The identification of the phenolic components was made by comparison of retention times and spectra with those of commercially available standard compounds. For the purpose of quantification, linear regression models were determined using standard dilution techniques.

Statistical Analysis. All samples were prepared and analyzed in triplicate. Results are reported as the mean ± standard deviation for 10 measurements on each animal group. To compare the three groups, analysis of variance (ANOVA) was used. Means were compared by least significant differences (LSD), and a probability of $P < 0.05$ was adopted as the criterion for significant differences.

Table 6. Radical-Scavenging Activity of Meat Samples Measured by DPPH$^{\bullet}$ (IC_{50}) and ABTS$^{\bullet+}$ Assays[a]

	IC_{50} (mg/mL)		μM Trolox/g of fresh meat	
	M. deltoideus	M. obliquus externus abdominis	M. deltoideus	M. obliquus externus abdominis
control	227.96 ± 35.552 a	187.02 ± 15.539 a	498.25 ± 94.305 a	481.78 ± 84.979 a
10%	157.99 ± 30.651 b	125.27 ± 32.648 b	1184.98 ± 196.401 b	1077.03 ± 186.619 b
20%	134.56 ± 58.556 b	117.21 ± 13.269 b	1030.64 ± 253.242 b	962.10 ± 96.128 b

[a] Values within columns with common letters were not significantly different ($P < 0.05$); ± standard deviation.

RESULTS AND DISCUSSION

The introduction of a new product (distilled rosemary leaves) in an animal diet implies the control of the animal productivity. Thus, daily lamb weight gain was measured from birth to slaughter to ensure that the animal production was not decreased during these assays. The results show that the introduction of rosemary leaves (10 or 20%) into the ewes' diet did not modify lamb production parameters, because the values were similar for the three groups: control (0.18 ± 0.087), 10% (0.18 ± 0.093), and 20% (0.19 ± 0.075) kg.

Meat Polyphenolic Profile. The occurrence of polyphenolic components in lamb meat has been studied by means of liquid chromatographic analysis applied to the methanolic extracts. The results obtained (**Tables 2** and **3**) show that there was a positive transfer of polyphenols to the lamb meat as a result of the dietary application of rosemary leaf byproduct to the ewe mothers under study. Chromatographic analysis allowed the identification of 11 polyphenolic components previously identified in the rosemary and basal diet pellets, respectively. Among them, rosmarinic acid, carnosol, and carnosic acid were the phenolic components that had a significantly increased presence in the lamb meats from the ewes fed this aromatic herb when compared to the control group. These results cannot be related to others previously published because, to the best of our knowledge, there is no literature regarding the analysis of polyphenolic compounds in lamb meat from animals fed rosemary leaves. Carnosic acid was the lipid-soluble diterpene quantified at the highest concentrations in samples of both muscles. Other polyphenolic components identified at higher concentrations in the rosemary leaf diets, including hesperidin and genkwanin, did not have an increased presence in the lamb meat analyzed. This can be attributed, as reported by O'Grady et al. (*23*), to the excretion of these components in the urine or perhaps to a biotransformation into unavailable forms in the ruminant digestive system.

Another factor that was evaluated in the present work was whether the incorporation of distillate rosemary leaves at different percentages in the sheep mother's diet affected the lamb meat polyphenolic quantitative profile. According to the results shown in **Tables 3** and **4**, no statistically significant differences were detected among the polyphenolic components quantified in both experimental groups (10 and 20%), except for carnosic acid in the meat from the muscle deltoideus. Probably, as stated before, the majority of these components were excreted in the urine. This is not the subject of the present work, but a deeper study should be made to learn how these components are metabolized by the ruminant digestive system.

Total Antioxidant Activity of Lamb Meat. To assess the effectiveness of the dietary administration of distilled rosemary leaves, the antioxidant status of lamb-meat samples was determined by means of their radical-scavenging (against the radicals DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) and total reducing (FRAP assay) activities. As reported by Descalzo et al. (*28*), the meat homogenates contained agents that reacted directly with TPTZ. Endogenous Fe^{II} in the meat homogenates could be responsible for the FRAP reaction. On the basis of this statement, the ferric reducing power of meat extracts is expressed as the difference between the FRAP and $FRAP_0$ values. The results in **Table 5** show that the administration of rosemary leaf distillate in the mothers' diets led to higher lamb-meat reducing potentials than those calculated for the control samples, as assessed by FRAP ($P < 0.05$).

The radical-scavenging capacity of the meat tissues was determined as inhibition of the autoxidation of ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals. According to the results shown in **Table 6**, statistically significant differences ($p < 0.05$) were detected between the groups fed the distilled rosemary leaves and the control group in this assay. As shown by the FRAP determinations, the incorporation of this byproduct into the animal diet favored the antioxidant stability of the meat samples, because lower IC_{50} (DPPH$^{\bullet}$) values were obtained for these tissues when compared with the control group. This quenching ability is also corroborated by the analysis of the radical-scavenging activities exhibited by the meat samples against the cation ABTS$^{\bullet+}$.

In the comparison of the results obtained from the two ewe groups fed rosemary leaves (10 or 20%), it is important to remark that no statistically significant differences were detected between the values of the two experimental groups. From this, it can be concluded that the incorporation of distilled rosemary leaves at 10% of the ewe diet should be enough to improve the meat's antioxidant status. This affirmation is in accordance with the results published by Nieto et al. (*30*). For these authors, feeding with 10 or 20% rosemary leaf improved the quality and shelf life of raw lamb meat packed in a modified atmosphere (70% O_2/30% CO_2) due to the fact that this delays the oxidation of fat and color, and microbiological spoilage, improving the appearance of the meat. However, O'Grady et al. (*23*) concluded that the supplementation of cattle diet with rosemary extract (1000 mg/animal /day) did not improve significantly the surface redness and lipid stability of the fresh beef. In this case, the concentration of extract added was not sufficient to improve the meat quality.

Descalzo et al. (*28*) determined the overall antioxidant status in fresh beef from pasture or grain-fed cattle. For these authors, fresh meat produced on pasture had higher total FRAP levels than meat from grain-fed animals; however, no differences were found regarding the ability to reduce ABTS$^{\bullet+}$. Petron et al. (*31*) reported the oxidative stability of lamb meat from animals grazing in pastures of different botanical composition. The results showed that pasture type significantly affected protein oxidation and the activity of glutathione peroxidase, but no significant differences were found for the α-tocopherol content, color, or lipid oxidation of Longisimus thoracis et lumborum muscle samples. These findings highlight the effectiveness of the distilled rosemary leaves, because an improvement in the meat's antioxidant status has been demonstrated, with regard to not only its reduction potential but also the free radical-scavenging capacity of the tissue homogenates.

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