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Relevance of the carnosic acid/carnosol ratio for the level of rosemary diterpene transfer and for improving lamb meat antioxidant status

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ABSTRACT

The aim of the present work was to evaluate whether the relation between the concentrations of the two major diterpenes present in two typified rosemary extracts affects their levels of deposition and antioxidant capacity in different lamb tissues. The composition of the extracts expressed as percentage of weight/weight was 1:1 (14–16)% and 2:1 (25–11)% (carnosic acid–carnosol), respectively. Thirty weaned lambs were assigned randomly to three homogeneous groups. One group was fed a basal diet as a control and the diets of the other two were enriched with rosemary extracts 1:1 and 2:1, respectively. HPLC–ESI-MS/TOF identified a metabolite ($C_{19}H_{22}O_3$) described for the first time in lamb tissues, along with carnosol, carnosic acid, rosmanol and carnosol-*p*-quinone. The results obtained corroborate the importance of the presence of carnosol in the dietary administration of rosemary extract as a way of improving the stability of the diterpene fraction during feed manufacturing and the level of deposition and antioxidant efficacy of diterpenes after ruminal fermentation.

1. Introduction

The demand by consumers for healthier foods obtained from sustainable and drugs-free farming systems is one of the most important reasons for the implementation of new feeding strategies by livestock producers (Font i Furnols et al., 2011).

The use of rosemary plants or the corresponding polyphenolic extracts in ewe and lamb feeding, as a way of increasing the shelf-life of the meat, has been addressed by several researchers (Bañón, Mendez, & Almela, 2012; Morán, Andrés, Bodas, Prieto, & Giráldez, 2012; Morán, Rodriguez-Calleja, et al., 2012; Morán et al., 2013; Nieto, Díaz, Bañón, & Garrido, 2010). For these authors, the introduction of carnosic acid in lamb feeding seems to be a useful way to delay lipid peroxidation, to improve colour and flavour stabilities along with meat texture and to protect against cholesterol oxidation.

The effectiveness of rosemary in meat antioxidant stability is mainly related to the presence in its polyphenolic composition of the diterpenes, carnosic acid and carnosol, and the caffeoyl compound, rosmarinic acid (Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008). However, controversy exists concerning the effectiveness of an antioxidant component when used in *in vitro* or *in vivo* assays. The direct antioxidant activity of a dietary compound depends on its absorption in the gastrointestinal tract and on its deposition in the tissues (Vasta & Luciano, 2011). Much about the intestinal mechanisms related with the gastrointestinal absorption of polyphenols in ruminants remains unknown.

The bioavailability of carnosic acid in lamb meat (Moñino et al., 2008), goat milk (Jordán, Moñino, Martínez, Lafuente, & Sotomayor, 2010) and plasma, liver and brain of rats (Doolaege, Raes, De Vos, Verhe, & De Smet, 2011; Romo et al., 2013) has already been tested.

Another interesting point worth considering is the possible interaction between exogenous antioxidants, like vitamin E, and polyphenolic metabolites in animal tissues. Gladine, Morand, Rock, Bauchart, and Durand (2007) reported that feeding rats with polyphenol-enriched diets lowered lipid oxidation and increased vitamin E levels in liver, compared to the control diet. It appears that dietary polyphenols might also exert an indirect antioxidant protection mediated by their direct role in restoring vitamin E from its oxidised form, or in protecting vitamin E from oxidation (Deckert et al., 2002). Therefore, if polyphenols act in synergy with other antioxidant systems, dietary strategies involving the use of plant or plant extracts rich in polyphenols should be carefully designed to consider the effect of the diet on the intake of other antioxidants and on the endogenous antioxidant systems.

Taking into account all these considerations, the supplementation of lamb diets with natural antioxidant compounds as an

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alternative to synthetic antioxidant additives, and as a means of improving lamb-meat quality and animal welfare merits investigation. To our knowledge, research into the use of rosemary extracts in lamb feeding, has mainly used extracts rich in carnosic acid, or the whole extract.

Nevertheless, how the relative concentration of these two major diterpenes of rosemary extract affects the level of transference of these components, as regards the antioxidant status of different lamb muscles and liver, has not been addressed.

The hypothesis is based on the fact that, as a potent antioxidant, carnosic acid exhibits low oxidative stability. In its oxidation pathway, carnosol is one of the main degradation products. The chemical equilibrium between the reactant (carnosic acid) and the final product (carnosol) modulates the stability of both components. In fact, according to the studies conducted by Zhang et al. (2012), when carnosic acid and carnosol were present in the same ethanolic solution, carnosic acid showed some protective capacity towards carnosol, and even increased the concentration of carnosol derived from carnosic acid. Based on the above, the relative concentration of these two diterpenes should affect their individual oxidative stability, although it is known that it does not affect their total antioxidant activities *in vitro* (Jordán, Lax, Rota, Lorán, & Sotomayor, 2012).

Following this line, the aim of the current study was to investigate these phenomena *in vivo*. Namely, whether including dietary rosemary extracts with different concentrations ratios of carnosic acid and carnosol in the diet affects the polyphenolic composition of lamb-meat and its antioxidant capacity.

2. Material and methods

2.1. Reagents

All the chemicals used were of AnalR grade. The 2,2-Diphenyl-1-picrylhydrazil (DPPH$^{\cdot}$), TPTZ (2,4,6-tripyridyl-s-triazine), $FeSO_4 \cdot 7H_2O$, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and potassium persulphate ($K_2S_2O_8$) were purchased from Sigma–Aldrich (Madrid, Spain). The 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-Holland). Petroleum ether and formic acid, analytical grade, were purchased from Scharlau Chemie S.A. (Barcelona, Spain). Pure standards of carnosol (COL), and carnosic acid (CA) were obtained from Sigma–Aldrich (Madrid, Spain).

2.2. Rosemary extracts

In this experiment two rosemary extract powders (30–36% of diterpenes HPLC purity) provided by Nutrafur S.A. (Murcia, Spain) were assayed. Both extracts were composed of a mixture of diterpenes (carnosic acid and carnosol) at different concentration ratios. The quantitative composition of extract 1 was (14–16)% and for extract 2 (25–11)% (carnosic acid–carnosol), respectively. These extracts were added as a further additive during feed manufacture at an initial concentration of 2700 ppm.

2.3. Animals and diets

Thirty weaned Segureño lambs (13 ± 1 kg) were assigned randomly into three homogeneous groups. All the animals were fed with fattening feed supplied *ad libitum* until they reached a live weight of 25 ± 2 kg. The fattening feed period lasted 56 ± 11 days. Table 1 shows the ingredients of the fattening feed for lambs provided by Nanta Animal Nutrition (Torre Pacheco, Murcia, Spain). The dietary treatments differed in terms of the relative concentrations of the antioxidant diterpenes used as a supplement. Standard feed manufacture conditions related to temperature and pressure during the whole period of pelleting (17 min) were in the range of 70–75 °C and 2 bar.

Table 1
Lamb feed composition.

Proximate composition (Dry-matter)	(g 100 g^{-1})
Crude protein	176
Ether extract (crude lipids)	38
Crude fibre	45
Crude ash	63
Total energy	(kcal kg^{-1})
	3911
Ingredients and additives	(g kg^{-1})
Wheat bran	89.4
Corn	200
Wheat	330
Molasses-cane	10.0
Soy husk	32.2
Malting barley	94.8
Fat	6.5
Calcium soap	3.1
Glycerine	10.0
Calcium carbonate	21.4
Sodium bicarbonate	5.0
Sodium chloride	3.0
Other minerals	3.0
	(IU kg^{-1})
Vitamin D3	1600
Vitamin A	8000
	(mg kg^{-1})
Vitamin E	25

Diet 1 was enriched with 640 ppm of diterpenes (carnosic acid/carnosol), diet 2 contained 685 ppm of these components, and the control treatment involved no additional supplementation. Differences in the final content of active components in the two diets are attributed to the different level of degradation of diterpenes during feed manufacturing.

The lambs were reared at the CIFEA Research Centre (Consejería de Agricultura, Región de Murcia, Spain).

After the rearing period, all the animals were slaughtered in a local abattoir according to EC Regulations. The carcasses were chilled at 2 °C for 72 h in a cooling room.

2.4. Meat and detoxification organs sampling

Fresh samples (72 h post-slaughter) of meat from the front legs (*M. deltoideus*) and the abdominal wall (*M. obliquus externus abdominis*) were cut into pieces, vacuum-packed, and stored at −80 °C prior to analysis. The selection of these meat pieces took into consideration consumer preference for lamb, since they are known to be the most flavourful cuts. At the same time, livers and kidneys were removed and stored in the same conditions as described above for the meat samples.

2.5. Diterpene extraction

Feed pellets were dried in a forced-air drier (P Selecta, Barcelona, Spain) at 35 °C for 48 h (until they reached a constant weight) and then ground to pass through a 2 mm sieve.