Determination of tocopherol and carotenoid contents in ST muscle of suckling lambs using fresh or lyophilised muscle

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Abstract. The aim of the study was to compare tocopherol and carotenoids contents in lyophilised and fresh meat to reduce the time and chemical reactives used in the analytical procedure. Twenty ewe-lamb pairs grazed on pastures (723, 570, 147 and 6.6 μg/g DM of β-carotene, lutein, α- and γ-tocopherol contents respectively; Grazing) and 19 pairs were raised indoorswith ewes receiving pasture hay (24, 100, 11 and 1.1 μg/g DM of β-carotene, lutein, α-, and γ-tocopherol contents respectively; Hay). After slaughter (live-weight: 11 ± 0.8 kg), both Semitendinosus muscles were excised to determine carotenoids and tocopherol contents in lyophilised or fresh meat. The contents of α-tocopherol and lutein determined using lyophilised meat were 2.8-fold and 21.3-fold greater than using fresh meat (P<0.001). The content of β-carotene was undetectable whereas γ-tocopherol content was not detected in lyophilised meat and near 0 in fresh meat. Regarding the effect of the ewe's diet, α-tocopherol content was greater in grazing than in hay treatment, regardless of the method of extraction (1.35 vs. 0.83 μg/g FM in lyophilised and 0.56 vs. 0.21 μg/g FM in fresh meat). Lutein content was greater in grazing than in hay treatment lambs in lyophilised meat (0.28 vs. 0.08 μg/g FM) but did not differ in fresh meat. The content of γ-tocopherol content was greater in grazing than in hay treatment lambs in fresh meat (0.003 and 0.001 μg/g FM, respectively, P<0.05). Consequently, it would be more appropriate to determine α-tocopherol and lutein contents in lyophilised meat, although γ-tocopherol content was not detectable.

Keywords. Lutein – Forage – Sheep – Carotenoids.

Détermination des tocophérols et caroténoïdes dans le muscle ST frais ou lyophilisé chez l'agneau allaité

Résumé. *L'objectif de l'étude consiste à comparer les teneurs des tocophérols et caroténoïdes entre le muscle frais et lyophilisé pour réduire le temps d'analyse et les réactifs chimiques employés. Pour aboutir à cet objectif on a utilisé 20 paires brebis-agneau alimentés sur pâturage (570, 723, 147 et 6,6 μg/g MS de lutéine, β-carotène, α-, γ-tocophérol respectivement ; pâturage) et 19 paires ont été alimentés à l'intérieur. Les brebis ont reçu le foin des prairies (24, 100, 11 et 1,1 μg/g MS de lutéine, β-carotène et α-tocophérol, respectivement ; (foin). Après l'abattage (poids vif : 11 ± 0,8 kg), les muscles Semitendinosus ont été extraits pour déterminer les caroténoïdes et tocophérols dans le muscle frais et le muscle lyophilisé. Les teneurs en α-tocophérol et lutéine déterminés au niveau du muscle lyophilisé ont été de l'ordre de 2,8 et 21,3 fois supérieur que ceux dans le muscle frais (P<0,001). La teneur en β-carotène a été indétectable alors que la teneur en γ-tocophérol n'a pas été détectée au niveau du muscle lyophilisé et était proche de 0 dans le muscle frais. Concernant l'effet du régime alimentaire des brebis, le teneur en α-tocophérol a été supérieur chez les agneaux Pâturage que chez les agneaux Foin dans le muscle lyophilisé (1,35 vs. 0,83 μg/g MF) et le muscle frais (0,56 vs. 0,21 μg/g MF). Le teneur en lutéine a été supérieur chez les agneaux Pâturage que chez les agneaux Foin au niveau du muscle lyophilise (0,28 vs. 0,08 μg/g MF) mais elle était similaire pour le muscle frais. Le teneur en γ-tocophérol a été supérieur chez les agneaux Pâturage que chez les agneaux Foin dans le muscle frais (0,003 et 0,001 μg/g MF, P<0,05). En conclusion, il est plus approprié de déterminer la concentration en α-tocophérol et en lutéine dans le muscle lyophilisé, cependant, le teneur en γ-tocophérol n'a pas été détectable.*

Mots-clés*. Lutein – Fourrages – Ovins – Caroténoïdes.*

I – Introduction

In dry mountain areas, grazing of ewe-lamb pairs to produce suckling lambs can be an alternative to indoors feeding of ewes (Joy *et al.*, 2012) to reduce production costs. Moreover, a percentage of lamb consumers prefer meat from grass-fed animals probably because the beliefs and expectations toward grass-fed meat are related with healthier, tastier, more natural and environmentally friendly meat (Font-i-Furnols *et al.*, 2011). However, consumers demand information on the traceability of the product (Bernues *et al.*, 2003). Thus, there is an increasing interest in guaranteeing the traceability of these production systems. The authentication of forage feeding in ovine meat can be achieved by measuring carotenoid pigments, lutein specifically (Prache, 2007). However, lutein content on fresh meat is analysed using liquid chromatography, being these analyses expensive and time-consuming. Muscle lyophilisation may reduce the quantity of chemical reactives and the time needed for the analyses. The aim of the study was to determine the content of carotenoids and tocopherols in meat of suckling lambs of grazing and indoors systems in lyophilised and fresh meat.

II – Materials and methods

1. Animals

This study was conducted in spring in La Garcipollera Research Station, located in the Spanish Pyrenees (Spain, 42º37' N, 0º30'W; 945 m a.s.l.). Twenty ewe-lamb pairs grazed on pasture (NDF: 44.6%; ADF: 18.5%; CP: 23.9%) from birth until slaughtering until lambs reached 10-12 kg liveweight. Nineteen ewe-lamb pairs were stalled and ewes were fed pasture hay (NDF: 63.3%; ADF: 33.8%; CP: 6.9%). Ewes received daily 300 g of concentrate. When lambs reached the target slaughter weight they were transported to CITA abattoir (180 km). Carcasses were chilled for 24 h.

2. Sampling

Samples of the different feedstuffs were collected weekly. They were immediately vacuum-packed and frozen until they were analysed. Samples were protected from light. The feedstuffs were thawed at room temperature the same day of the analysis.

Both Semitendinosus (ST) muscles of each animal were extracted and immediately vacuum-packed in opaque packages and frozen. Thereafter, one muscle per animal was freeze-dried. Muscles were weighed before and after freeze-drying to estimate dry matter content. Both muscles were kept at -80ºC until analyses. The day of the analyses both muscles were thawed at room temperature and minced.

3. Analyses of carotenoids and tocopherol concentrations

The contents of β-carotene, lutein and α-tocopherol in the feedstuffs were determined by HPLC following the procedures of Val *et al.* (1994). The contents in muscle were analysed according to Lyan *et al.* (2001) with modifications: meat (0.1 g of lyophilised meat and 0.4 g fresh meat) and ethyl alcohol (0.4 and 1 ml for lyophilised and fresh meat, respectively) were mixed and vortexed for 30 s. One ml of n-hexane was added and the mixture was vortexed for 15 min, centrifuged at 3.500 rpm for 5 min at room temperature. The hexane phase was collected. The extraction with 1 ml of n-hexane was repeated. The hexane phases of both extractions were evaporated with a vacuum centrifuge. The dry residue was dissolved in 1 ml µl of methanol. The content in lyophilised meat was determined similarly, except that carotenoids and tocopherols were determined by UHPLC (Acquity H-Class, Water, Milford, USA) with a 100×4.6 mm, RP C_{18}, 2.1 µm Kinetex column and krudkatcher ultra HPLC in-line filter. Lutein and β-carotene were detected with a photodiode

array detector at 450 nm and tocopherols by fluorescence at L*ex* 293 nm and L*em* 322. The isocratic mobile phase was methanol (0.05% triethanolamine). All results obtained in fresh and lyophilised meat were expressed as µg/g of fresh matter (FM).

III – Results and discussion

Average carotenoids, α- and γ-tocopherol contents of the feedstuffs used in the experiment are detailed in Table 1. The major carotenoid in pasture was β-carotene followed by lutein while lutein was the major carotenoid in pasture hay. Regarding tocopherols, α-tocopherol was the major tocopherol in all the feedstuffs. Pasture had the greatest carotenoids contents because hay making decreases carotenoids (Nozière *et al.*, 2006). The concentrate had no carotenoids because high temperatures during processing probably destroy the low quantities present in corn or other components of the concentrate (Nozière *et al.*, 2006).

Table 1. β-carotene, lutein, α-tocopherol and γ-tocopherol concentrations in the feedstuffs

	Pasture	Hay	Concentrate
β-carotene, µg/g DM	723.0 ± 233.4	23.7 ± 19.4	n.d.
Lutein, µg/g DM	569.5 ± 165.1	100.0 ± 33.5	1.0 ± 1.1
α-tocopherol, µg/g DM	147.4 ± 85.1	10.9 ± 6.3	9.3 ± 5.4
γ-tocopherol, µg/g DM	6.6 ± 5.4	1.1 ± 1.1	6.2 ± 1.5

n.d.: not detected.

Regarding the effect of the feeding system, lambs whose dams grazed had greater α-tocopherol content both in fresh and lyophilised muscle (P<0.001) than lambs whose ewes were hay-fed indoors (Fig. 1) as reported by D'Alessandro *et al.* (2012) in the *Semimembranosus* muscle of suckling lambs. Grazing lambs had greater lutein content than Hay lambs in lyophilised (P=0.04) but similar content in fresh muscle (P=0.41) (Fig. 1). Grazing lambs had greater γ-tocopherol content than Hay lambs in fresh muscle (0.003 vs. 0.001 µg/g FM; P=0.03) but the content was not detected in lyophilised muscle. Suckling lambs, with maternal milk as unique food, had lower γ-tocopherol in *Longissimus dorsi* and leg muscles than lambs fed milk replacer (Osorio *et al.*, 2008). The milk replacer, enriched with tocopherols, had greater tocopherols contents than maternal milk.

The method of extraction affected lutein, α- and γ-tocopherol contents in muscle (Table 2). The contents of α-tocopherol and lutein in lyophilised muscle were 2.8-fold and 21.3-fold greater than in fresh muscle (P < 0.001), respectively. The content of γ-tocopherol was only detectable in fresh muscle while β-carotene content was not detected in fresh or lyophilised muscle. Freeze-drying increased lutein content in egg yolk compared to fresh matter (Wenzel *et al.*, 2010). These authors proposed that lyophilisation led to an irreversible denaturation of (lipo)proteins followed by a release of associated xanthophylls that had not been previously extractable.

Table 2. Effect of the method of extraction on carotenoids and tocopherols contents in the *Semitendinosus* muscle

	Fresh	Lyophilised	RSD	P-value
Luteín, µg/g FM	0.011	0.234	0.11	0.002
β-carotene, µg/g FM	n.d.	n.d.	–	–
α-tocopherol, µg/g FM	0.38	1.08	0.23	0.001
γ-tocopherol, µg/g FM	0.002		0.003	–

n.d.: not detected.

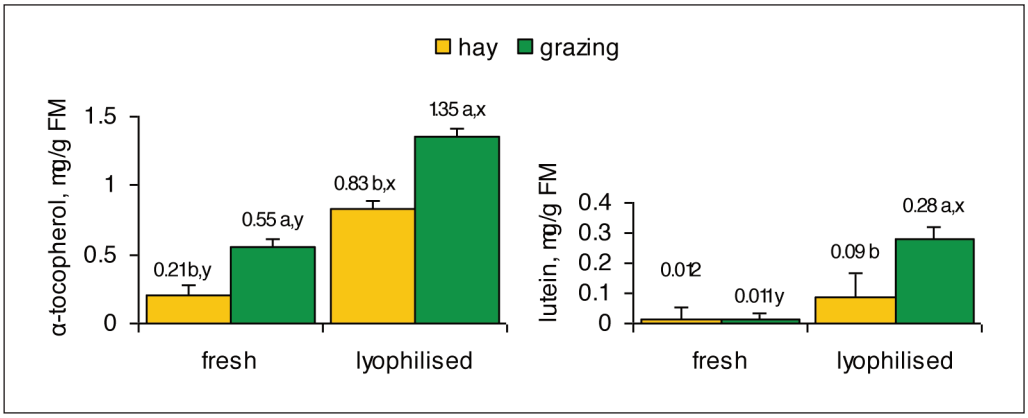

Fig. 1. Effect of the feeding system (hay vs. grazing) on α-tocopherol and lutein content of lyophilised and fresh meat of ST muscle in suckling lambs. Hay: suckling lambs whose ewes were fed hay indoors; grazing: suckling lambs that grazed with their dams at pasture. Within a parameter; a,b indicate differences due to the feeding system at P<0.05; x,y indicate differences due to the method of extraction at P<0.05.

IV – Conclusions

Lutein and α-tocopherol contents in muscle could be used as markers of grazing in ovine due to the greater contents in pasture than in hay-fed animals, however more studies should be carried out to confirm these results. The determination in lyophilised muscle was more appropriate than in fresh muscle because it showed differences due to the feeding system in lutein content in addition to α-tocopherol content.

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