Journal of Food Composition and Analysis 36 (2014) 59–65

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

# Effect of pasture and concentrate diets on concentrations of carotenoids, vitamin A and vitamin E in plasma and adipose tissue of lambs

R. Álvarez [a], A.J. Meléndez-Martínez [b,*], I.M. Vicario [b], M.J. Alcalde [a]

[a] Dept. Agricultural and Forestry Science, Universidad de Sevilla, Seville, Spain
[b] Food Colour & Quality Laboratory, Dept. of Nutrition and Food Science, Universidad de Sevilla, Seville, Spain

ARTICLE INFO

*Article history:*
Received 15 May 2014
Received in revised form 16 July 2014
Accepted 5 August 2014
Available online 28 August 2014

*Keywords:*
Lamb
Meat quality
Retinol
Tocopherol
Carotenoids
Reflectance spectrum
Vitamin A
Vitamin E
Livestock practices and nutritional composition
Traceability in the food chain
Food composition
Food analysis

ABSTRACT

The effect of different diets on the content of carotenoids, fat-soluble vitamins (retinol and tocopherol) and color measurements in sheep tissues was assessed, to study the possible links with the livestock production system for both nutrition and traceability. Three groups of animals were considered: 15 lambs fed on pasture (G1), 15 lambs fed on concentrate (G2) and 15 suckling lambs (G3) whose mothers were grass-fed. Lutein and β-carotene appeared only in plasma from grazing lambs, so they appear useful to differentiate lambs fed on pasture. Furthermore, retinol and α-tocopherol content in fat were significantly higher ($p < 0.01$) in the animals fed on an extensive system ($1.48 \pm 0.08$ and $42.4 \pm 1.76$ μg/g fat, respectively) than those fed on an intensive one ($1.03 \pm 0.08$ and $34.8 \pm 0.23$ μg/g fat, respectively). When fat-soluble vitamin (retinol and tocopherol) levels and the absolute value of the integral from the reflectance spectrum, between 450 and 510 nm, from fat samples were used in a discriminant model, 100% of the lambs were correctly classified according to their feeding system (pasture vs. concentrate).



## 1. Introduction

Consumers are becoming more health-conscious and there is a trend toward healthy and nutritious foods with more health-promoting functions (Olmedilla-Alonso et al., 2013). In this sense, consumers are increasingly focusing both on the "green image" and the health-promoting properties of products from animals fed on pasture (Dian et al., 2007a; Nozière et al., 2006a; Prache et al., 2003b; Sheath et al., 2001). For these reasons, research on diet authentication in herbivore products is conducted within a general context of increasing consumer awareness concerning the mode of animal production (Dian et al., 2007a). In this regard, certain compounds from the diet, such as carotenoids, have been used to authenticate production system (outdoors vs. indoors) of animal products (Dian et al., 2007b; Prache et al., 2003a; Prache, 2009; Serrano et al., 2007; Sheath et al., 2001). Despite outdoor and indoor production systems, it has been reported (Alcalde et al., 2013; Ripoll et al., 2012) that the most widely used system in small ruminant farming in Mediterranean countries involves suckling kids and lambs, which are slaughtered at low live weights of approximately 10–12 kg. Therefore it seems useful to evaluate the existence or not of correlations between the levels of carotenoids and other micronutrients in the plasma and milk of the ewe and the plasma of the suckling lamb as a possible means to authenticate the exclusive milk diet of these animals.

Mammals are not able to synthesize vitamin A (retinol) and E (mainly in the form of α-tocopherol), so these vitamins must be provided by the diet. Provitamin-A carotenoids (mainly β-carotene) and tocopherol are abundant in fresh pastures and forage, whereas concentrate feedstuffs tend to present very low levels of these compounds, since oxidation may occur during manufacture (Dunne et al., 2009; Nozière et al., 2006a; Ramírez and Quiles, 2005; Schweigert, 1998). Furthermore, the role of fat-soluble vitamins in the nutritional and sensory properties of foods has been recently pointed out (Sauvant et al., 2011), so these vitamins would be significant for consumers not only

* Corresponding author at: Food Colour & Quality Lab., Dept. of Nutrition & Food Science, Universidad de Sevilla, Facultad de Farmacia, 41012 Sevilla, Spain. Tel.: +34 954557017.
*E-mail address:* ajmelendez@us.es (A.J. Meléndez-Martínez).

http://dx.doi.org/10.1016/j.jfca.2014.08.001
0889-1575/

for traceability purposes, but also from the nutritional point of view.

Besides the presence of carotenoids in tissues, other techniques have been developed, in order to discriminate pasture-fed animals from animals with a diet based on concentrate. A mathematical analysis of fat reflectance as an indirect system has been proposed to estimate the concentration of carotenoids (Prache and Theriez, 1999), in order to discriminate two production systems (extensive vs. intensive) in herbivores. This method has been validated in sheep in a large cohort of animals (Dian et al., 2007a). However, the carotenoid concentration in animal tissue can range widely according to dietary supply (Dian et al., 2007b). So, it would be interesting to seek for new compounds and/or methodologies that could be used to differentiate feeding systems individually or in combination with those already developed, in order to increase the degree of discrimination.

In consequence, the aims of this study were to assess the effect of different diets on the carotenoids and fat-soluble vitamin levels of retinol and α-tocopherol in different sheep tissues, and to study the possible links with the livestock production system, for both nutrition and traceability. Another objective was to evaluate if their use in combination with color measurements was useful to discriminate pasture-fed from stall-fed lambs. Finally, the possible existence of correlations between the levels of fat-soluble vitamins and carotenoids of the ewe and the suckling lamb was also studied.

## 2. Material and methods

### 2.1. Animals and diets

Three groups of lambs, all from the *Merino* breed, were considered for this study. Group 1 (G1) included 15 male light lambs, which were weaned at 45 days old and reared in an extensive system with a diet based on pasture until the moment of slaughter (around 90 days old), when the animals had an average weight of 21.05 ± 1.02 kg. The paddock where the lambs were reared was in the southwest of Spain, an area characterized by a *dehesa* landscape, i.e. a forest of oaks, cork oaks and other species from different *Quercus spp*., alongside grassland. Specifically for this paddock the pasture was mainly composed of plants from *Poaceae* family, such as *Agrostis spp*., *Vulpia spp*., *Poa spp*., *Bromus spp*. and *Lollium spp*. Species from *Fabaceae* family were also present; like *Trifolium spp*., *Ornithopus spp*., *Medicago spp*. and *Lotus spp*. Animals from group 2 (G2) were also 15 light lambs weaned at 45 days and fattened in an intensive system until slaughter (around 90 days old). These animals had a diet based on concentrates, composed of barley (40.1%), maize (30.0%), wheat (4.0%), soy (22.0%), fat (1.0%), calcium carbonate (1.6%), salt (0.3%), sepiolite (0.5%), flavoring (0.1%) and a corrector for minerals (0.4%). At slaughter these lambs has an average weight of 24.79 ± 0.52 kg. Finally, group 3 (G3) consisted of 15 suckling lambs (around fifteen days old) with an average live weight of 7.18 ± 0.23 kg. Their mothers were grass-fed with a supplementation of oat ad libitum as whole grains. They were reared in a paddock in the southwest of Spain, in a *dehesa* forest too; therefore the main composition of pasture was similar to that in group 1. Water and salt blocks were always available.

### 2.2. Sampling

Representative samples of the different diets were taken in triplicate from the farms and analyzed. In the case of pasture samples, the sampling areas were randomly selected where the animals were reared. The pasture was sampled from the ground; to do this, three sampling squares (0.5 m by 0.5 m) were randomly established. Finally, pasture samples were characterized overall according to the majority herbaceous species present at genus level. After collecting, all food samples were transported to the laboratory, lyophilized and stored at −80 °C until analysis. In the case of milk samples (G3), 20 mL of milk were collected from each ewe in the second week of lactation. These samples were also transported to the laboratory and stored at −80 °C until analysis.

Lambs from groups 1 and 2 were slaughtered in spring according to the European Regulation (CE No. 1099/2009 of 24 September 2009). Animals arrived at the abattoir by truck the night before slaughter and had access to water until 30 min before slaughtering. The slaughterhouse was 110 km from the pasture paddock (G1) and 74 km from the stall (G2). At the moment of slaughter blood samples were taken from each animal using Li-Heparin as anticoagulant. In addition, in the course of carcass dressing immediately after slaughter, 3 g of adipose tissue from the perirenal area, i.e. from the top of the kidney, of each animal were also collected. This location was selected as previous authors (Priolo et al., 2002) reported that the difference between feeding treatments was significantly higher for perirenal than for subcutaneous caudal fat in lambs, supporting the hypothesis of a greater accumulation of carotenoid pigments in perirenal compared to caudal fat in lambs fed grass. One hour after slaughter, the instrumental color measurements of the perirenal fat samples were carried out. Then, the samples were transported to the laboratory at 4 °C, blood was then centrifuged (1500 × *g*, 10 min, 4 °C), and the plasma was collected. All samples were stored at −80 °C until further analysis.

With respect to suckling lambs from group 3 and their mothers, blood samples were taken at the farm from the jugular vein of each animal, using Li-Heparin as anticoagulant. In this case, blood samples were also transported to the laboratory at 4 °C to centrifuge them (1500 × *g*, 10 min, 4 °C) and collect the plasma, which was stored at −80 °C until analysis. Prior to the analysis, all samples were thawed overnight in the dark at 4 °C.

### 2.3. Instrumental color measurements

The perirenal fat *CIELAB* (CIE, 1986) color parameters ($C^*_{ab}$, $L^*$, $a^*$, $b^*$ and $h_{ab}$) were measured on a CM-700d spectrocolorimeter (Konica Minolta Holdings, Inc., Osaka, Japan), using a $D_{65}$ illuminant, the 10° observer and zero and white calibration. Reflectance spectra in the visible region (between 360 and 740 nm, with 10 nm increments) were also acquired and recorded in order to obtain translated reflectance values ($TR_i$) and the absolute value of the integral (*AVI*) of the translated spectra. In previous studies (Priolo et al., 2002; Ripoll et al., 2008; Zawadzki et al., 2013) the reflectance spectra between 510 and 450 nm were translated to make the reflectance value at 510 nm equal to zero (*TR*). The translated reflectance values ($TR_i$) were calculated from the reflectance values ($R_i$) as follows: $TR_i = R_i - R_{510}$, with $I = 360$, 370, 380, …, 740; whereas *AVI* of the translated spectra were calculated according to the following formula;

$$AVI = [(TR_{450}/2) + TR_{460} + TR_{470} + TR_{480} + TR_{490} + TR_{500} + (TR_{510}/2)] \times 10$$

### 2.4. Carotenoids, retinol and α-tocopherol extractions

#### 2.4.1. Diets samples

Pasture and concentrate samples were analyzed in triplicate according to the methodology described previously (Kean et al., 2007; Panfili et al., 2004) with some modifications. One gram of each sample was lyophilized prior to the analysis. The extractions were carried out on the lyophilized extracts with a hexane/ethanol mixture (1:1, v/v) as extractant. Two extractions with 3 mL were