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Case study of a commercial sheep flock under extensive mountain grazing: Pasture derived lipid compounds in milk and cheese

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ABSTRACT

Terpenoid, fat-soluble antioxidant and fatty acid (FA) composition of pasture as well as those of milk and cheese from a commercial sheep flock managed under extensive mountain grazing in the east region of the Cantabrian mountain (Northern Spain) was investigated. The grazing period lasted for 2 months and ewes were at late lactation stage. Plants, feces, bulk milk and cheese samples were collected on two sampling dates. The abundance of the dominating botanical families in the mountain pasture prevailed in the sheep diet of the commercial flock. Major terpenoids and tocols in the pasture appeared as major ones in milk and cheese, whereas C18 unsaturated FAs in milk and cheese were derived from the intake of C18 polyunsaturated FAs which were prevalent in the pasture. No carotene was detected in the dairy samples but retinol (free or esterified), derived from the intake of β-carotene present in pasture plants, was found in milk and cheese.

1. Introduction

Currently there is an increasing interest in animal foods derived from mountain grazing. Apart from their valuable contribution to livestock production, mountain grasslands contribute to biodiversity conservation, maintenance of landscapes and mitigation of pollution. The Cantabrian mountain area (Northern Spain) has historically supported an important grazing-based sheep production, but a progressive abandonment of extensive grazing is expected in the short to medium term due to factors such as low farm profitability, scarce mountain facilities and low generational replacement (Rounsevell, Annetts, Audsley, Mayr, & Reginster, 2003). As in other mountain areas of southern Europe, sheep milk and cheeses are traditional foods, perceived by most consumers and producers themselves as high-quality foods. Therefore, on-farm investigations are necessary to provide data from the real context of mountain farms in order to relate extensive grazing management to milk and cheese quality.

Mountain pastures show a great diversity of plant species which, in turn, contain a rich variety of terpenoids, tocopherols and other compounds, depending on plant species, geographical, agronomic and climatic factors (Caballero et al., 2009; Cornu et al., 2001). A few studies with experimental cow herds have indicated that the lipid composition of milk and cheese was related to mountain pasture plant composition (De Noni & Battelli, 2008; Falchero et al., 2010). The increase in α-tocopherol in milk fat from grazing cows and goats as compared with indoor concentrate and forage-fed animals has been reported in a few cases (Delgado-Pertíñez, Gutiérrez-Peña, Mena, Fernández-Cabanás, & Laberye, 2013; Marino et al., 2012), whereas comparatively more attention has been paid to the FA composition of milk and cheese from mountain grazing cows in experimental studies (Povolo et al., 2012; Revello Chion et al., 2010). However, systematic studies relating lipid compounds present in pasture plant species to the lipid composition of milk and cheese from cows ingesting those plants are very scarce (Collomb, Bütikofer, Sierber, Jeangros, & Bosset, 2002). To the best of our knowledge, no such studies have been conducted with commercial sheep flocks under commercial

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milk and cheese production conditions. Thus, the objective of this study was to investigate the possible relationship between terpenoids, tocopherols, carotenoids and FAs in pasture plants to those present in the actual sheep diet, and finally to the milk and cheese composition from a commercial sheep flock under extensive grazing on Cantabrian mountain grasslands.

2. Materials and methods

2.1. Study area and commercial flock

The study was conducted in the Aralar Natural Park (42° 59′ 48″ N and 2° 06′ 51″ W) which is an 11,000 ha protected area located in the Basque Country (Northern Spain). Mean annual temperature is 12.4 °C, and mean annual precipitation exceeds 1400 mm. It has oceanic climatic conditions. The vegetation in the Park comprises a mosaic of gorse-heather shrublands and grasslands, which support livestock mainly 18,000 dairy sheep of *Latxa* breed managed in an extensive grazing system. The area traditionally used by livestock (beef cattle, dairy sheep and horses) occupies 2077 ha in the Park (18.9%), and its utilization also varies seasonally (from May to November), although the sheep milking period goes from early May to the end of June. The *Jasiono-Danthonietum* grassland occupies 1173 ha (56.5% of the grazing area) and the annual average primary production ranges from 1720 kg dry matter (DM)/ha to 3370 kg DM/ha depending on the year, precipitation rate and site. The stocking rate ranges between 2.3 and 4.5 livestock units (ha/day) (Mendizabal, 2009; Odriozola, García-Baquero, Laskurain, & Aldezabal, 2014).

One commercial sheep flock with around 150 lactating *Latxa* ewes participated in this study. Flock was allowed to graze all day (free grazing) from early May to the end of June in a grazing area of 42 ha, with around 7 km perimeter and nearly 500 m unevenness. The farm was located at 850 m altitude and ewes walked around 7–10 km *per* day (depending on the environmental conditions). Every day ewes remained overnight in a 1 ha grassland next to the farm before the morning milking.

2.2. Botanical composition of the grazing area

Previous knowledge of the study area (Mendizabal, 2009) was used to define the grazing area of the commercial flock. After that, a vegetation map was created and the relative abundance of each vegetation type in the grazing area was calculated using a Geographic Information System (GIS). The botanical composition of the pasture in the grazing area was estimated in two steps. First, the relative abundance of plant species within a vegetation type was estimated as previously described (Mendizabal, 2009). Second, the abundance (i.e. availability) of each plant species within the overall grazing area was calculated weighing its abundance with the percentage of occupation of each vegetation type in the grazing area. Finally, plant species were grouped into botanical families and their relative contribution to the native mountain pasture was calculated.

Seventeen plant species were sampled in the grazing area: *Festuca rubra*, *Agrostis capillaris*, *Poa annua*, *Lolium perenne*, *Luzula campestris*, *Carex caryophyllea*, *Trifolium repens*, *Lotus corniculatus*, *Ulex europaeus*, *Hippocrepis comosa*, *Bellis perennis*, *Achillea millefolium*, *Potentilla montana*, *Thymus praecox*, *Cerastium fontanum*, *Erica vagans* and *Ranunculus bulbosus*. The selection criterion was the relative abundance of botanical species available in the mountain pasture (Mendizabal, 2009). Individual samples of each plant species were taken on the second week of May and June. To collect a representative sample, at least 20 individual items of each plant species were picked up manually, including leaves, stems and flower parts, in a random walk through the grazing area. Around 25 g (fresh matter) of each botanical species were collected separately in plastic bags, hermetically sealed and transported in the dark to the laboratory in a portable cooler with ice. Once in the laboratory, samples were vacuum-packed and stored in darkness in a freezer at −35 °C. The samples were lyophilized at −60 °C and <6 Pa for 48 h. Freeze-dried samples were ground, stored in sealed zipper plastic bags and kept in a desiccator in the dark at room temperature until analysis. All analyses were done in duplicate. Dry matter (DM) was determined as described previously (AOAC, 2000).

2.3. Sheep diet composition

A total of 20 individual fecal samples were collected to determine by microhistological analysis the diet composition of the commercial flock (10 samples in May and 10 in June). Sampling dates were the same as those for plant species collection, and feces were collected in the morning in the place where ewes remained overnight. Feces were analyzed as previously described (Sparks & Malechek, 1968), and a reference collection of cuticles of the plant species present in the study area was prepared. Cuticle fragments of fecal samples were preserved in acetic-formaldehyde (analytical grade, Panreac, Madrid, Spain) until analysis. For each fecal sample, three sub-samples of the preserved fecal material were examined under the microscope at ×100 magnification. Cuticle fragments were quantified by counting all the identifiable fragments found in two longitudinal transects of 40 mm across the microscope glass slide, aiming for at least 230 identifiable plant fragments *per* sample ($Range_{May}$ = 249–320, $Mean_{May}$ = 277; $Range_{June}$ = 238–307, $Mean_{June}$ = 264). More than 200 cuticle fragments *per* sample were identified in order to ensure a good characterization of the individual diet. Taking into account that the microhistological technique is a very time consuming method, similarity indexes for fecal samples in each month were calculated to estimate the reliability of the results. Similarity indexes were calculated as $1 - B$ where B was the Bray–Curtis index of dissimilarity (Krebs, 1999). Similarity indexes for May and June samples were 83.9 ± 5.8% and 88.1 ± 3.4%, respectively, and therefore, it was considered that 10 fecal samples *per* month were enough to estimate the sheep diet composition of the flock. Identified plant cuticles were grouped into botanical families and the relative percent abundance *per* individual sample was estimated. Results were expressed as mean values of percentage of relative abundance.

2.4. Milk and cheese sampling

Bulk raw milk samples (1.5 l) from the manual morning milking were taken twice a month (replicate samples on consecutive weeks) from the second week of May and June (n = 4). Dry matter (DM) mean percentage (by weight) of milks was 19.44 ± 0.66, and total fat and protein mean percentages in DM were 43.27 ± 4.34 and 50.97 ± 0.52, respectively. Commercial cheeses were manufactured according to the PDO Idiazabal guidelines (Ministerio de Agricultura, Pesca y Alimentación, 1993). The sheepherder made cheese in a 200 l vat with the same bulk milk sampled, and one cheese was randomly collected *per* vat after 150 days of ripening (n = 4). Cheeses were made and ripened in the mountain farm facilities using manual vat and ripening chamber without temperature and relative humidity control. Milk samples and cheeses were transported in a portable cooler with ice. Once in the laboratory, aliquots of whole milk of each sample were stored in 50-ml screw-capped plastic pots at −80 °C and cheeses were cut in sections (~200 g), vacuum-packed and frozen at −35 °C, until analysis. DM mean percentage of cheeses was 67.48 ± 4.23, total fat and protein mean percentages in DM were 51.42 ± 7.18 and