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Changes in terpenoid composition of milk and cheese from commercial sheep flocks associated with seasonal feeding regimens throughout lactation

Izaskun Valdivielso, Mertxe de Renobales, Noelia Aldai, and Luis Javier R. Barron[1]
Lactiker Research Group, Faculty of Pharmacy, University of the Basque Country, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain

ABSTRACT

Changes in the terpenoid content of milk and cheese from commercial sheep flocks monitored throughout lactation in the Cantabrian area of northern Spain were investigated. The flocks followed the same seasonal feeding strategy during lactation: indoor feeding in winter (early lactation) based on concentrate and forage; part-time grazing in the valley in early spring (mid lactation); and from mid spring on (late lactation), flocks were managed under extensive mountain grazing. In the present study design, seasonal feeding and lactation stage were intrinsically linked and could not be considered in isolation, and a holistic approach was necessary to consider the whole production management of the commercial flocks studied. Furthermore, the study focused on the identification of sesquiterpenoid ratios to differentiate milks and cheeses produced under extensive mountain grazing from those produced under other seasonal feeding regimens. Total abundance of mono- and sesquiterpenoids and that of individual compounds such as α-pinene, β-caryophyllene, α-humulene, α-amorphene, and γ-cadinene significantly increased in milk and cheese from indoor feeding to mountain extensive grazing. Sesquiterpenoid ratios such as γ-cadinene/α-muurolene, γ-cadinene/δ-cadinene, β-caryophyllene/α-muurolene, and (β-caryophyllene + γ-cadinene)/α-muurolene were used to differentiate mountain milks and cheeses from those from indoor feeding and part-time grazing in the valley. Multivariate discriminant analysis applied to individual terpenoids and sesquiterpenoid ratios showed milk and cheese samples classified into 2 groups: samples from indoor feeding and part-time grazing in the valley were classified together, and clearly separated from mountain milks and cheeses. The results of the present study showed that the sesquiterpenoid ratios approach could help to differentiate mountain dairy products from others obtained under other specific feeding regimens in a local environment.

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[1]Corresponding author: luisjavier.rbarron@ehu.eus

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INTRODUCTION

Consumers have a growing interest in the nutritional and sensory quality of the food products that they purchase. In addition, regarding foods of animal origin, the concern extends to environmental issues, animal welfare, and production conditions, with special focus on the animal diet (Bernués et al., 2003; Sivadier et al., 2008). Consumers show an increasing preference for food products of pasture-based production systems. Dairy and meat products are involved in this consumer demand, in particular, those made from milk or meat from animals managed under traditional systems such as extensive mountain grazing. In different countries, essentially in Europe, these foods are protected by different types of specific labels. However, major difficulties arise when it comes to authenticate grassland products, or to discriminate these products from others obtained from different animal feeding regimens based on concentrates and forages. Among these challenges are the seasonal and geographic variation of the composition of the grasslands and the detection of non-grass feedstuffs in a grassland production system (Moloney et al., 2014).

Although research has been done to characterize milk and cheese from grazing animals, there is still much to do to differentiate grassland products. Most studies focused on identifying in milk and cheese direct or indirect molecular markers of grazing management such as fatty acids, terpenoids, tocopherols, carotenoids, and other compounds (Prache et al., 2005). Terpenoids originate from the secondary metabolism of plants and are mostly transferred directly from the ingested plant to the milk and cheese (Viallon et al., 2000; Bouvier et al., 2005) although some authors found that terpenoids can be degraded or modified by rumen bacteria and lactic acid bacteria used for cheesemaking (Broudiscou et al., 2007; Belviso et al., 2011). Also, high variability in the terpenoid composition of grazing milk and cheese occurs mostly due to pasture plant diversity,

geographical grassland altitude and location, seasonal feeding variations linked to lactation stage and pasture availability, and type of management systems (Cornu et al., 2001; Fernández et al., 2003; Tornambé et al., 2006; Agabriel et al., 2007; De Noni and Batelli, 2008; Revello Chion et al., 2010; Abilleira et al., 2011). Despite the variability, some specific terpenoids such as β-caryophyllene, α-copaene, α-pinene, and β-pinene are often found in milks and cheeses from animals grazing in humid pastures of grasslands in Europe (Tornambé et al., 2006; Revello Chion et al., 2010; Borge et al., 2016; Valdivielso et al., 2016b), and even β-caryophyllene has been proposed as a potential pasture-diet marker because of its ubiquity in fresh pasture plants (Mariaca et al., 1997; Favaro et al., 2005; Abilleira et al., 2011; Valdivielso et al., 2016b). On the other hand, terpenoids can contribute floral and vegetal odor to the flavor of cheese made with milk from grazing animals (Bugaud et al., 2001; Carpino et al., 2004). However, in general, small differences in the sensory profile of cheeses made with milk from animals fed concentrate, or mixed rations, in comparison with those made with milk from grazing herds were reported (Cornu et al., 2009; Valdivielso et al., 2016a).

Regarding the authentication of grassland products, the use in feedstuffs of various plant materials that can also provide several of the same compounds as fresh grass does is an important problem. In this respect, approaches involving the use of methodologies that use information from different markers or ratios between them could be effective (Renna et al., 2012; Povolo et al., 2013). This study addresses the changes in the terpenoid composition of milk and cheese associated with seasonal feeding regimens during lactation of commercial sheep flocks located in the Cantabrian area of northern Spain: indoor feeding, part-time grazing in the valley, and extensive mountain grazing. In the present study design, seasonal feeding and lactation stage were intrinsically linked and could not be considered in isolation, and a holistic approach was necessary to consider the whole production management of the commercial flocks studied. Furthermore, the study focused on the identification of sesquiterpenoid ratios to differentiate milks and cheeses produced under extensive mountain grazing from those produced under other seasonal feeding regimens.

MATERIALS AND METHODS

Commercial Flocks and Feeding Management

Six commercial flocks of Latxa dairy sheep belonging to the Protected Denomination of Origin (**PDO**) Idiazabal cheese (Basque Country region in northern Spain) were selected. Each flock had 200 to 500 lactating ewes during the study period and suckling lambs were weaned at 30 d, whereas milking period was extended from February (early lactation) to late June (late lactation). The study was conducted in 2011. Same feeding regimen was followed by all shepherds; from February to mid March, sheep were given concentrates and conserved forages (alfalfa and grass hay), from late March to late April sheep grazed part-time with concentrate and forage supplementation, and from May on sheep were moved to protected mountain grasslands (11,000 ha) in Aralar Natural Park (42°59′48″N and 2°06′51″W). These mountain grasslands are traditionally divided (no physical boundaries) in several pasture areas that are independently used by each flock. These areas showed the same vegetation type as detailed below. Up to 20 flocks belonging to the PDO Idiazabal cheese are moved every year to this natural park where shepherds have their mountain farms to make cheese. The 6 commercial flocks selected for this study grazed in different pasture areas of Aralar Natural Park. The concentrates and conserved forages included in the feeding regimens were purchased from local suppliers by shepherds and some even prepared their own forages. Feedstuffs in concentrates were mainly soy, corn, wheat, barley, oilseed flour, and in some cases, beet pulp, whereas alfalfa and grass hay were the most commonly used conserved forages. During indoor feeding, each ewe was fed with 400 to 1,500 g/d of concentrate and 4,000 g/d of forage in fresh matter. Thus, among shepherds the concentrate to forage ratio varied from 0.2 to 3.0. During part-time grazing, ewes were allowed to graze up to 8 h/d depending on weather conditions in cultivated private pastures where ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) were predominant, or in community-owned noncultivated grasslands that consisted of several herbaceous species such as *Trifolium repens*, *Festuca rubra*, and *Agrostis capillaris*, with other nongraminoid plants and shrubs (Mandaluniz et al., 2009). In this period, each ewe was supplemented with 400 to 1,000 g/d of concentrate and with no forage or with 200 to 1,500 g/d of conserved forages. The concentrate to forage ratio during part-time grazing varied among shepherds from 0.3 to 2.0. During mountain grazing, sheep flocks were kept outdoors and were allowed to graze all day long. The same specific pasture area was used by each flock during early and late mountain grazing. The vegetation type and abundance of botanical species of Aralar Natural Park have been previously reported (Valdivielso et al., 2016b). Jasiono-Danthonietum grassland (code 6230, subtype a) is the most abundant vegetation showing a rich plant biodiversity with over 30 different botanical families of which *Poaceae*, *Fabaceae*, *Juncaceae*, and *Asteraceae*