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# Effect of the inclusion of fresh lemon pulp in the diet of lactating ewes on the properties of milk and cheese





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# ABSTRACT

This study investigated the effects of fresh lemon pulp (FLP), as a natural antioxidant in the diet, on the intake of feed and the production of milk and cheese of Valle del Belice lactating ewes during the hot summer in Sicily. A total of 15 second-lambing ewes, kept individually in  $3 \times 3$  m pens, were divided into 3 homogeneous groups fed with 3 diets in a  $3 \times 3$  Latin square design, with 3 experimental phases of 21 days each. The diets were: mixed hay ad libitum plus 600 g/day of concentrate (FLP0); mixed hay ad libitum plus 400 g/day of concentrate and 1 kg/day of FLP (FLP1); and mixed hay ad libitum plus 200 g/day of concentrate and 2 kg/day of FLP (FLP2). Nine experimental Pecorino cheeses were manufactured with bulk milk collected at the end of each phase from each group. The ewes of each group showed the same dry matter (DM) intake (2 kg/day/head), but the FLP2 group received lower (P = 0.001) net energy for lactation (NE<sub>1</sub>) than other groups (2.13, 2.36, and 2.31 Mcal/day per head for FLP2, FLP0, and FLP1, respectively). The FLP constituted 9% and 16% of the total DM intake in the FLP1 and FLP2 groups, respectively. In general, the daily milk yield was low, reflecting the effect of the high environmental temperatures, and was lower (P = 0.001) in the FLP2 group than in the other groups (323, 355, and 369 g/day for FLP2, FLP1, and FLP0, respectively), probably due to the lower daily energy intake. Milk protein (P = 0.046) and casein (P = 0.033) percentages were higher in the FLP2 group than in the FLP1 group; the FLP-fed groups had higher levels of (P = 0.011) milk urea than the FLPO group, due to a higher (P = 0.001) CP/NE<sub>L</sub> ratio in the ingested diet (96.4, 95.8, and 95.3 g/Mcal for FLP2, FLP1, and FLP0, respectively). The fatty acid composition of milk from FLP2-fed ewes was higher in vaccenic (10.6 vs. 7.96 mg/g fat; P = 0.031) and rumenic acids (6.21 vs. 5.30 mg/g fat; P = 0.048) than that in milk from FLP0 ewes. The characteristics of the cheeses were not influenced by the diet, with the exception of the total content of phenolic compounds (P = 0.011) and antioxidant activity (P = 0.051), both of which were higher in cheeses made with milk from FLP-fed ewes.

## 1. Introduction

Citrus fruits are consumed by humans principally as fresh fruit or processed juice, either fresh or reconstituted from concentrate. The residues that remain after the juice is extracted from the fruit comprise peel (flavedo and albedo), pulp (juice sac residue), rag

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Abbreviations: BPF, citrus by-product feedstuffs; FLP, fresh lemon pulp; GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity; FA, fatty acids; FAME, fatty acid methyl esters; CLA, conjugated linoleic acid; VA, vaccenic acid; RA, rumenic acid; SCC, somatic cell count

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(membranes and cores), and seeds. These components, either individually or in various combinations, are the source materials from which citrus by-product feedstuffs (BPF) are produced. Citrus BPF are suitable energy sources for ruminant feed in many areas of the world (Bampidis and Robinson, 2006) because ruminants are able to ferment highly fibrous feeds in the rumen. An important benefit of citrus BPF–based feeding is its relatively low cost. Reduction of feed costs, while maintaining high productivity, is a primary strategy to achieve economic efficiency in ruminant production.

Citrus BPF contain a variety of energy substrates, including soluble carbohydrates and rapidly degradable NDF made up of cellulose and pectin. Because of these nutritive characteristics, which have fewer negative effects on the rumen ecosystem than supplementation with starch or sugar-rich feeds, citrus BPF can be used as a high-energy feed to support growth and lactation in ruminants (Bampidis and Robinson, 2006).

Citrus pulp is usually fed dehydrated; however, it can also be fed fresh or as silage, although fresh citrus pulp is generally transported only for short distances because of its high moisture content and high transportation costs (Bampidis and Robinson, 2006). Fresh citrus pulp can meet some of the water requirements of ruminants, which can be important in areas characterised by hot, dry summers. The use of dried and ensiled citrus pulp for ruminant nutrition has been studied extensively (Bampidis and Robinson, 2006; Volanis et al., 2006), whereas studies on fresh citrus pulp fed to sheep appear to be limited. Among citrus BPF, lemon pulp is less readily available than orange pulp, but it shows particular characteristics that make it more suitable for fresh consumption; in fact, the lower level of residual sugars, which often supports secondary fermentation and/or mould growth, and the lower pH contribute to its preservation (Bampidis and Robinson, 2006). Both lemon pulp and orange pulp are generally very rapidly accepted by ruminants, but pulp and peels from lemons are somewhat more acceptable than those from oranges and grapefruit (Bath et al., 1980). The higher antioxidant properties of lemons (total polyphenols, essential phenols, ascorbic acid) compared to other citrus fruits, such as oranges and grapefruits (Gorinstein et al., 2001), also make them preferable for the prevention of cancer and degenerative diseases in human nutrition (Tripoli et al., 2007).

Few papers have reported on the utilisation of fresh citrus pulp in the diet of dairy sheep. Studies have examined the effects of whole citrus fruits (Clementines) on lactating ewes and found analogous effects on ruminal parameters between whole citrus fruits and fresh citrus pulp (Piquer et al., 2009) and no negative effects on milk (Jaramillo et al., 2009; Piquer et al., 2011) and cheese production (Jaramillo et al., 2009). A dietary integration of 2 kg/day per head or ad libitum administration of fresh lemon pulp (FLP) has been reported for dairy ewes in Sicily, and their effects on milk yield and composition (Scatassa et al., 2006; Todaro et al., 2006) and cheese characteristics (Chiofalo et al., 2004; Todaro and Scatassa, 2011) have been evaluated. These studies have seen no negative effects on milk production, instead highlighting increased milk yield in dairy ewes fed with FLP ad libitum (Todaro et al., 2006). The effects on cheese characteristics regarded mainly the flavour, attributed to the higher presence of limonene and  $\beta$ -pinene in Pecorino cheeses made with milk from FLP-fed ewes (Chiofalo et al., 2004), while some problems linked to acidification of the curd were observed in the production of *pasta filata* cheese made from raw milk, as in the Vastedda della valle del Belice Protected Designation of Origin cheese (Todaro and Scatassa, 2011).

In Sicily, a greater amount of fresh lemon pulp is available in early summer, when the by-product can be properly used to compensate for the shortage of fresh forage in the diet of dairy ewes, which are in an advanced stage of lactation at that time. Accordingly, the objective of the present study was to evaluate the effects of different levels of FLP fed to late-lactation ewes in summer, on the basis of their feed intake, in terms of milk and cheese yield and quality, with particular regard to the fatty acid (FA) composition of milk and the polyphenol enrichment and antioxidant capacity of cheese.

## 2. Material and methods

#### 2.1. Animals, experimental design, and diets

The experiment was conducted at the farm of the Istituto Sperimentale Zootecnico per la Sicilia (Sicilian Region, Palermo, Italy) for a period lasting 10 weeks (June to August 2015). During the experimental period, minimum environmental temperatures ranged from 20 °C to 27 °C and maximum temperatures were between 28 °C and 37 °C. At the farm, 15 second-lambing Valle del Belice ewes in late lactation were selected and assigned to 3 groups that were homogeneous in terms of days in milk (155  $\pm$  12 days), body condition score (2.36  $\pm$  0.18) and daily milk yield (372  $\pm$  119 g/day). The ewes were housed in individual 3  $\times$  3 m pens during the experimental period. After a 2-week period of adaptation to these housing conditions, the 3 groups of ewes were fed 3 different diets in succession, according to a 3  $\times$  3 Latin square design, with each phase composed of 14 days of adaptation to the diets and 7 days for data and sample collection (sampling week).

The 3 experimental diets were based on the same vetch-oats hay offered ad libitum, integrated with 600 g/day of commercial concentrate only (FLP0), 400 g/day of commercial concentrate plus 1 kg/day of fresh lemon pulp (FLP1), or 200 g/d of commercial concentrate plus 2 kg/d of fresh lemon pulp (FLP2). In practice, therefore, 1 kg of FLP replaced 200 g of the commercial concentrate.

#### 2.2. Sampling and analysis

At the beginning and end of each experimental phase, all ewes were weighed and monitored for their body condition score (BCS) according to Russel et al. (1969). During each sampling week, all offered and refused feeds of each ewe were weighed and sampled daily to estimate the amount and quality of feed intake.

The samples of mixed hay, commercial concentrate, and FLP were pooled for each sampling week and analysed following the procedures of the AOAC (2005) to determine the concentrations of dry matter (DM, 934.01), ash (942.05), crude protein (CP,

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