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Milk fatty acid composition, rumen microbial population, and animal performances in response to diets rich in linoleic acid supplemented with chestnut or quebracho tannins in dairy ewes

A. Buccioni,* M. Pauselli,† C. Viti,* S. Minieri,* G. Pallara,* V. Roscini,† S. Rapaccini,* M. Trabalza Marinucci,‡ P. Lupi,* G. Conte,§ and M. Mele§

*Dipartimento di Scienze delle Produzioni Agro-alimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 18, 50144 Firenze, Italy
†Dipartimento di Scienze Agrarie Alimentari ed Ambientali, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
‡Dipartimento di Medicina Veterinaria, University of Perugia, Via S. Costanzo 4, 06126 Perugia, Italy
§Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

ABSTRACT

The aim of the study was to evaluate milk fatty acid (FA) profile, animal performance, and rumen microbial population in response to diets containing soybean oil supplemented or not with chestnut and quebracho tannins in dairy ewes. Eighteen Comisana ewes at 122 ± 6 d in milking were allotted into 3 experimental groups. Diets were characterized by chopped grass hay administered ad libitum and by 800 g/head and day of 3 experimental concentrates containing 84.5 g of soybean oil/kg of dry matter (DM) and 52.8 g/kg of DM of bentonite (control diet), chestnut tannin extract (CHT diet), or quebracho tannin extract (QUE diet). The trial lasted 4 wk. Milk yield was recorded daily, and milk composition and blood parameters were analyzed weekly. At the end of the experiment, samples of rumen fluid were collected to analyze pH, volatile fatty acid profile, and the relative proportions of *Butyrivibrio fibrisolvens* and *Butyrivibrio proteoclasticus* in the rumen microbial population. Hepatic functionality, milk yield, and gross composition were not affected by tannin extracts, whereas milk FA composition was characterized by significant changes in the concentration of linoleic acid (CHT +2.77% and QUE +9.23%), vaccenic acid (CHT +7.07% and QUE +13.88%), rumenic acid (CHT −1.88% and QUE +24.24%), stearic acid (CHT + 8.71% and QUE −11.45%), and saturated fatty acids (CHT −0.47% and QUE −3.38%). These differences were probably due to the ability of condensed versus hydrolyzable tannins to interfere with rumen microbial metabolism, as indirectly confirmed by changes in the relative proportions of *B. fibrisolvens* and *B. proteoclasticus* populations and by changes in the molar proportions of volatile fatty acids. The effect of the CHT diet on the milk FA profile and microbial species considered in this trial was intermediate between that of QUE and the control diet, suggesting a differential effect of condensed and hydrolyzable tannins on rumen microbes. Compared with control animals, the presence of *B. fibrisolvens* increased about 3 times in ewes fed CHT and about 5 times in animals fed QUE. In contrast, the abundance of *B. proteoclasticus* decreased about 5- and 15-fold in rumen liquor of ewes fed CHT and QUE diets, respectively. The use of soybean oil and a practical dose of QUE or CHT extract in the diet of dairy ewes can be an efficient strategy to improve the nutritional quality of milk.

Key words: tannin, milk fatty acid, sheep, microbial population

INTRODUCTION

During the last decade, several efforts have been done to enhance the level of healthy FA in milk and dairy products with the aim of improving the nutritional quality of foods deriving from ruminants (Chilliard et al., 2007; Mele, 2009). This objective may be achieved by applying feeding strategies based on dietary supplementation with polyunsaturated marine or vegetables oils or oilseeds (Shingfield et al., 2013), to accumulate in the rumen conjugated linoleic acid (*trans*-11,*cis*-9 CLA) precursors such as *trans*-11 18:1 (vaccenic acid, **VA**), and to increase the duodenal passage of PUFA. Previous studies demonstrated that adding vegetable oils rich in linoleic acid (*cis*-9,*cis*-12 18:2; **LA**) oil in the diet of small ruminants increased the content of *cis*-9,*trans*-11 CLA and VA in milk fat 2- to 3-fold (Mele et al., 2006, 2008; Gómez-Cortés et al., 2008). However, because the extent of rumen biohydrogenation (**BH**) of PUFA is usually more than 80 to 90%, the amount of supplemented lipid needed to achieve an effective enhancement of *cis*-9,*trans*-11 CLA and VA in milk fat from sheep and goats ranges from 60 to 100 g/head and

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[1]Corresponding author: arianna.buccioni@unifi.it

day, leading to an increase in feeding costs. Moreover, although small ruminants are less sensitive than dairy cows to milk fat depression syndrome, high levels of lipids coupled with low forage diets may induce a decrease in milk fat content (Bauman and Griinari, 2003; Shingfield et al., 2013). As a consequence, in the last years, increasing interest has been devoted to feed ingredients able to slow the extent of rumen BH of dietary PUFA to obtain significant accumulations of VA in the rumen and, thus, an increase of the transfer of this FA from rumen to duodenum and then to the mammary gland (resulting in an increase of *cis*-9,*trans*-11 CLA, which originates by mammary desaturation of VA), while using lesser amounts of lipid supplementation.

Several in vitro studies have demonstrated that tannins are able to interfere with rumen BH or methane production, according to their polyphenolic nature (Bhatta et al., 2009; Khiaosa-Ard et al., 2009; Buccioni et al., 2011). Moreover, ewes and cows fed diets containing less than 4% tannins on a DM basis resulted in higher retention of nitrogen and lower plasma urea concentrations, because of the ability of tannin to protect feed protein from rumen microbial degradation (Frutos et al., 2004a). The effect on rumen microorganism activity has been related to the ability of tannins to interfere with the membranes of rumen bacteria, binding enzymes or by deprivation of metal ions, such as iron (Patra and Saxena, 2011). Among bacterial species involved in BH processes of PUFA, *Butyrivibrio fibrisolvens* and *Butyrivibrio proteoclasticus* seem to be the most sensitive (Vasta et al., 2010), but specific studies on the effects of different types of tannins on rumen microbial population are still scarce. Moreover, results from in vitro and in vivo experiments often show conflicting results on the effect of tannins on the accumulation of BH intermediates in the rumen and on the productive response of the animal (Vasta et al., 2009a; Toral et al., 2011, 2013). This is probably due to differences in tannin species and percentage inclusion in the diet and to associative effects between tannins and other diet ingredients such as lipids.

The aim of the present study was to evaluate the effect of moderate amount (<2%) of chestnut or quebracho tannin extracts (hydrolyzable and condensed tannins, respectively) in diets supplemented with soybean oil on the milk FA profile and on the relative abundance of *B. fibrisolvens* and *B. proteoclasticus* in the rumen microbial community. Moreover, because sheep milk is mainly used for cheese making, a further objective of the present study was to evaluate the effect of tannin addition on the gross composition and clotting characteristics of milk. Finally, because tannins may exert a toxic effect in ruminants, causing necrosis of the liver and lesions in the digestive tract (Reed, 1995; Hervás et al., 2003a), this experiment studied the effect of these feeding strategies on blood parameters, with a special focus on indicators of hepatic function.

MATERIALS AND METHODS

Experimental Design

Animals. Eighteen multiparous Comisana ewes at 122 ± 6 DIM kept at the Experimental Section of the Department of Applied Biology, University of Perugia Italy, were allotted into 3 experimental groups, homogeneous for BW (68.1 ± 7.83 kg), and each group was kept in multiple pens (6 ewes for each pen). The trial lasted 4 wk after 15 d of adaptation to the feeding regimen. The handling of the animals was according to Institutional Animal Care and Use Committee of the University of Perugia. The ewes were milked twice daily at 0730 and 1730 h using a milking machine (43 kPa; 150 pulsations/min), and daily individual milk yield was recorded.

Diets. The experimental diets were formulated according to the nutrient requirements of a ewe weighing 68 kg and producing 1 kg of milk at 6.5% fat (Cannas et al., 2004). Diets were composed of chopped grass hay (particle size >4 cm in length) administered ad libitum and 800 g/head and day of a concentrate that contained 84.5 g of soybean oil/kg of DM and 52.8 g/kg of DM of bentonite (control diet, **CON**), or 52.8 g/kg DM of chestnut tannins (**CHT** diet) or 52.8 g/kg DM of quebracho tannins (**QUE** diet). The chemical composition of feeds and the ingredients of concentrates are presented in Table 1. The dose of tannins was chosen to obtain a tannin concentration in the diet of almost 1.6% of expected DMI. On the basis of results from previous studies in literature, this dose was considered safe for the animal and practical for farmers (Hervás et al., 2003a,b; Frutos et al., 2004a,b). The experimental concentrates were offered after each milking, and 100 g/head of rolled barley was offered during milking. Chestnut hydrolyzable tannins (750 g/kg DM of tannic acid equivalent; by Gruppo Mauro Saviola srl Radicofani, Siena, Italy) and extract of quebracho tannins (456 g/kg DM of tannic acid equivalent; by Guido Lapi spa Castel Franco di Sotto, Pisa, Italy) were titrated according to Burns (1963).

Sampling and Analysis

Feed Sampling and Analysis. Samples of feeds and orts were weekly collected and stored at −80°C until analysis. Samples were freeze-dried and then ground for chemical analysis using a Cyclotec 1093 mill (PBI International, Milan, Italy) using a mesh size of 1 mm.