Livestock Science 123 (2009) 230–240

Contents lists available at ScienceDirect

Livestock Science

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

Responses to condensed tannins of flowering sulla (*Hedysarum coronarium* L.) grazed by dairy sheep
Part 2: Effects on milk fatty acid profile

A. Cabiddu *, G. Molle, M. Decandia, S. Spada, M. Fiori, G. Piredda, M. Addis

AGRIS Sardegna, Dipartimento per la Ricerca nelle Produzioni Animali, 07040 Olmedo, Italy

ARTICLE INFO

Article history:
Received 9 April 2008
Received in revised form7 November 2008
Accepted 17 November 2008

Keywords:
Hedysarum coronarium
PEG
Milk
Sheep
OBCFA
CLA
Tannins

ABSTRACT

A grazing experiment was undertaken to evaluate the effect of PEG supplementation on the fatty acid composition of milk from Sarda sheep grazing sulla. Twenty-four late-lactating sheep (12 per group), were paired and split into two groups: group control (CON), dosed daily with a quenching gun with 200 ml of water, and group PEG, dosed with 200 ml of a 50/50 w/v water solution of PEG. The sheep grazed two 0.8 ha plots of sulla under a rotational grazing scheme. The contents of *c-9, t-11* CLA and *t-11* C18:1 in milk fat were on average 40% higher ($P<0.01$) in the PEG group than in the CON group. This can be explained by the higher biohydrogenation activity of ruminal bacteria in the PEG group, due to the partial inactivation of the tannins. Odd-branched chain fatty acids (OBCFA) were higher in PEG than in the control group (+20%; $P<0.01$) and this confirms the hypothesis that tannin in sulla reduced ruminal microbial activity. Both linoleic (C18:2 *c-9 c-12*) and linolenic (C18:3 *c-9 c-12 c-15*) fatty acids were lower ($P<0.05$) in milk from PEG, than in the CON-group (−12% and −30% for linoleic and linolenic acids, respectively). The mitigating effect on tannins of PEG increased the ratio of ω6/ω3 by 24%; ($P<0.01$) and total *trans* FA content in milk by 20% ($P<0.01$). In conclusion, condensed tannins in sulla at flowering are conducive to lower *c-9, t-11* CLA and *t-11* C18:1 but also lower total *trans* FA, ω6/ω3 ratio and higher linoleic and linolenic acid.

1. Introduction

There is renewed interest in forage legumes, because of their important role in sustainable feeding systems. They are able to fix nitrogen and have potentially a high feeding value for ruminants (Rochon et al., 2004).

Sulla (*Hedysarum coronarium* L.) is a legume with a high nutritive value (Terrill et al., 1992; Molle et al., 2003). It usually contains a moderate level of condensed tannins (CT, 20–40 g kg DM^{-1}). These compounds have beneficial effects on the net uptake of amino-acids and parasite burden resilience in certain circumstances, depending, among other factors, on their concentration and source (Mueller-Harvey, 2006). By contrast, in other circumstances they have negative effects on ruminant nutrition because they reduce voluntary feed intake (VFI), proteins, structural carbohydrates digestibility and intestinal enzymes activity, or they cause illness (e.g. Silanikove et al., 1994). Reduction of diet digestion is attributed to the formation of stable complexes between CT and protein or/and carbohydrates, even though the great diversity of tannin in nature means that it is difficult to generalise about their effects. Indeed, some tannins cause lesions in the gut mucosa (Robbins et al., 1991), but can be degraded. Others cause rapid reduction of VFI through the emetic mechanism of the nervous system (Provenza et al., 1990). Tannins may decrease DM digestibility through their bacteriostatic and bactericidal effects on rumen microbes (Henis et al., 1964). Inhibition increases with the increase in the degree of tannin polymerization. The effects of CT on the growth of rumen bacteria and on microbial proteolysis have been recently described by Min et al. (2005), who clearly showed the reduction of the rate of proteolysis and the

* Corresponding author. Tel.: +39 079 387236; fax: +39 079 389450.
E-mail address: acabiddu@agrisricerca.it (A. Cabiddu).

1871-1413/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2008.11.019

inhibition of the growth of proteolytic rumen microrganisms. Attempts have been made to deactivate tannins with the use of polyethylene glycol (PEG), a non-nutritive synthetic polymer which has a greater binding affinity than proteins, makes tannins inert by forming tannin-PEG complexes (Makkar, 2003). Administration of PEG to sheep fed tannin-containing sulla at flowering (CT: 72 g kg DM^{-1}) increased crude protein (CP) and DM digestibility (Stienezen et al., 1996). These results were probably due to improvements in the rumen fermentation parameters, and in particular to an increase in the cellulase digestibility of rumen bacteria, with a consequent rise in short chain fatty acids (SCFA) and in the ammonia concentration in the rumen (Kobeisy et al., 1999).

In recent decades milk and dairy products have often been claimed to have detrimental effects on human health, because their consumption has been associated with high levels of coronary heart disease (CHD). This has been explained by the connection between the intake of *trans* fatty acids (TFA) and the risk of CHD. However recent estimates of average daily intake of TFA from animal products in the US population shows that on average they amount to only 0.53% of daily energy intake (US Food and Drug Administration, 2003). Most TFA from milk and dairy products consist of vaccenic acid and CLA (which have anti-carcinogenic, anti-atherogenic and anti-diabetic, body composition and immune function modulating properties), but it is undeniable that animal products also contain other potentially noxious TFA. On the other hand there is little doubt that maximizing omega-3 (ω3) fatty acid in ruminant products benefits human nutrition and health. Due to increased consumer awareness of the link between diet and health, recent research has focused on altering the FA composition of cow milk (Shingfield et al., 2005) or sheep milk (Kitessa et al., 2003) through supplementation with fish oils or other fish by-products. However only modest enrichment of ω3 content in milk fat has been achieved so far (Lock and Bauman, 2004). The Mediterranean basin is characterized by the bio-diversity of its grassland, which contributes to the distinct nature of its animal products. In this area, where dairy sheep nutrition is mainly based on grazing, improving the healthiness of dairy products through pasture management could be of interest. Fresh forages are important sources of ω3 FA, and in particular of linolenic acid (Cabiddu et al., 2006b).

In a previous study (Cabiddu et al., 2005) showed clearly how different forage species can increase significantly the level of polyunsaturated fatty acid (PUFA) and CLA in sheep milk. However, Addis et al. (2005) remarked that the phenological stage of some forage species, likewise sulla, can influence the trend of milk FA composition. In particular these authors linked the low level of CLA in sheep milk, with the rise of tannin content in sulla during spring (reproductive stage of forage). Piluzza et al. (2000) found a highest value of sulla tannins during the flowering stage, and Molle et al. (2003, 2004) showed how phenological stage influenced the level of condensed tannins and total phenols in fresh sulla. Since tannins are considered as "antinutritional factors" effort in the past has been to improve their nutritional value and to reduce the negative effect on animal performance. In particular as reported by Silanikove et al. (1994) supplementation with PEG to goats fed with carob leaves (2% of total phenols on DM basis) improved crude protein digestion and cell wall digestion (+50% respect to the control) in agreement with Decandia et al. (2008). Therefore as reported by Silanikove et al. (2001) PEG supplementation is very affective for to neutralize the effect of tannins also when total phenols level are around 2% of DM.

While the effect of PEG on nutrition and performance of sheep meat grazing sulla has been already addressed (Terrill et al., 1992), there is a lack of information on its effect on dairy sheep, and in particular fatty acid composition of the milk. Since the use of PEG involves an increase in ruminal biohydrogenation it is probable that the inactivation of tannins increases the biohydrogenation intermediates, conjugated linoleic acids (CLA) and vaccenic acid (VA), but at the same time decreases the level of PUFA in milk. This hypothesis is supported by the results of Turner et al. (2005), who showed that cows grazing birdsfoot trefoil had increased concentrations of saturated fatty acids and decreased ω3 fatty acids when supplemented with PEG. An experiment was carried out to test this hypothesis on dairy sheep grazing sulla. It was focused on a sulla pasture at the flowering stage, since previous studies had shown that the CT content of sulla peaked during that phase of the growing cycle (Molle et al., 2004).

The specific objective of the study was to investigate the effect of sulla CT on the fatty acid composition in the milk of sheep grazing sulla during the flowering stage. The effects on feeding behaviour, intake, digestibility and overall performance are reported in detail and discussed in a companion study (Molle et al., 2009).

2. Materials and methods

The experiment was conducted at the Bonassai research farm in spring 2004 (April–May, the flowering stage of the pasture). The details of pasture, chemical analysis and sheep management have been described previously (Molle et al., 2009). In brief, twenty-four lactating sheep, 162 ± 5 days in milk (means ± SEM) were randomly allocated to the following treatments: control (CON) dosed daily with 200 ml of water and PEG (4000 mol wt; Masnata Chimici S.p.A. Cagliari, Italy), dosed with 200 ml of a 50/50 w/v water solution of polyethylene glycol. Dosing was made at milking, twice daily (7:30am and 15:30pm). The groups grazed as a flock during the whole day with the exception of milking. No supplement was offered.

2.1. Fatty acids composition of herbage

Hand-plucked herbage samples were taken during the experimental period. These samples were freeze dried and their fatty acid composition analysed. Lipid extraction was carried out using a chloroform: methanol (2/1; v/v) method described by Christie (1989). Fatty acids were determined according to the method of Chin et al. (1992), using acid-catalysed methanolysis. Separation and quantification of methyl esters were carried out using a gas chromatograph (Varian 3900; Varian, Harbor City, CA), equipped with a split/splitless injector and a flame ionisation detector. Methyl ester separation was carried out on a capillary column SP2560 (100 m × 0.25 mm i.d., 0.25 μm of phase; Supelco Inc., Bellefonte, PA) using helium as the carrier gas (constant flow, 1 ml/min). The injector and detector temperature were set at 290 °C. The injection was carried out in split mode with