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Milk production, composition, and milk fatty acid profile from grazing sheep fed diets supplemented with chestnut tannin extract and extruded linseed

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ABSTRACT

Tannins are bioactive compounds able to interfere with protein and lipid metabolism in the rumen, by forming undegradable complexes with dietary proteins and by modulating several bacterial activities, including the biohydrogenation of polyunsaturated fatty acids. The aim of this trial was to study the effect of dietary supplementation with chestnut hydrolysable tannin extract on ewes milk yield and quality. Ninety-six multiparous Sarda ewes in their mid-lactation phase were allotted to two homogeneous groups (control group, C group; group fed concentrate supplemented with chestnut tannin extract, CHE group), each of 48 animals, for a feeding trial. Animals of both groups grazed 8 h per day on the same pasture based on a mixture of *Lolium multiflorum*, *Avena sativa* and *Trifolium repens* (1:1:1). The two diets differed only in their concentrate supplement. The control group received 450 g/head per day of a concentrate feed without chestnut tannin extract, whereas the CHE group received 500 g/head per day of a concentrate feed formulated with the same ingredients of the control concentrate plus 80.0 g/kg DM of chestnut tannin extract. The amounts of concentrate offered to the animals of both groups were calculated in order to obtain isoproteic and isoenergetic dietary treatments considering the expected DM intake of animals. The inclusion of chestnut tannin in the concentrate resulted in a greater production of milk (+18.64%; $P < 0.001$). Moreover, no differences in casein fraction profile between milks from both groups were found while the casein index was greater ($P = 0.034$) in milk from ewes fed CHE than milk from ewes fed C. As regard fatty acid composition, milk from CHE group had a greater concentration of omega-3 fatty acids if compared to milk from C group (alpha-linolenic acid: 2.18 *vs* 2.57 g/100 g of total lipids in C and CHE group, respectively), whereas the percentage of CLA and of C18:1 *trans*11 in milk fat from CHE group was smaller (CLA: 2.20 *vs* 1.85 g/100 g of total lipids, in C and CHE group, respectively with $P = 0.001$; C18:1 *trans*11: 3.89 *vs* 3.57 g/100 g of total lipids in C and CHE group, respectively with $P = 0.001$). The use of practical doses of CHT in the diet of grazing ewes may improve the response to dietary linseed supplementation, resulting in milk with a greater concentration of alpha-linolenic acid.

1. Introduction

In the Mediterranean area, during the early spring, the diet of lactating ewes is almost exclusively based on pasture, which is particularly rich in highly fermentable carbohydrates and proteins. In some cases, this feeding regimen is associated with an increase of urinary nitrogen losses and of elevate concentrations of urea in milk and blood, which, in turn, may be associated with decreased health status of sheep (Morgante, 2004). On the other hand, grazing is also usually associated with great contents of conjugated linoleic acid (CLA) and alpha-linolenic acid (α-LNA) in milk fat, especially in the early vegetative phase of pasture (Mele, 2009; Nudda et al., 2005).

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; NDF, neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; BH, biohydrogenation; C, control concentrate; CHE, experimental concentrate; CHT, chestnut tannin; C group, control group; CHE group, experimental group; CI, casein index; CLA, conjugated linoleic acid; DM, dry matter; DMI, dry matter intake; FA, fatty acid; FCM, fat corrected milk; α-LNA, alpha-linolenic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acids.

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However, the concentrations of CLA and α-LNA in milk fat may vary according to the pasture plant composition and to the length of the grazing activity (Cabiddu et al., 2005). In order to obtain milk with a stable fatty acid profile suitable for the production of cheese with proven positive effect on human health (Pintus et al., 2013), dietary lipid supplementation is also considered an effective feeding strategy. In particular, the inclusion of extruded linseed in the diet of dairy ewes resulted in an increase of CLA and α-LNA concentration in milk fat similar or greater than that reported for grazing ewes (Gomez-Cortes et al., 2009; Mele et al., 2011). The use of extruded linseed during the grazing period should promote an additive effect between the α-LNA contained in the fresh herbage and that contained in linseed. It could result in greater concentrations of CLA and α-LNA in milk fat than those reported for grazing ewes or for non-gazing ewes fed diet supplemented with extruded linseed. However, previous studies reported that extruded linseed supplementation in the diet of grazing ewes or cows did not result in an increase of α-LNA content in milk fat, suggesting that the α-LNA contained in linseed was almost completely biohydrogenated in the rumen (Addis et al., 2009; Lerch et al., 2012).

Tannins are bioactive phenolic compounds widely distributed in plant kingdom, which interfere with protein and lipid metabolism in the rumen, by forming undegradable complexes with feed proteins and by modulating the biohydrogenation (BH) of polyunsaturated fatty acids (PUFA; Buccioni et al., 2012; Minieri et al., 2014). Tannins may differ in their solubility and other chemical and physical characteristics, and also differ in their capacity to bind feed proteins or to influence the activity of rumen microorganisms (Carreño et al., 2015; Frutos et al., 2004a,b). In ruminants, tannins can have beneficial effects if they are present in the diet at moderate concentrations (Patra and Saxena, 2011). In fact, when ewes and cows were fed on diets containing less than 4% of tannins on dry matter basis, they had higher retention of nitrogen and lower plasma urea concentrations, as a consequence of the ability of tannin to preserve feed protein from rumen microbial degradation (Frutos et al., 2004a,b; Min et al., 2003). A recent study on dairy cows reported that hydrolysable tannins extracted from chestnut wood positively affected animal health, by inhibiting lipid peroxidation and by increasing antioxidant enzymes activities in plasma and liver, without worsening their milk production (Liu et al., 2013). Moreover, the efficacy and selectivity of chestnut tannins (CHT) in limiting BH extent of dietary unsaturated fatty acids, without detrimental effects on rumen microbiome, have been proven by *in vitro* trials (Buccioni et al., 2011). However, *in vivo* effects of hydrolysable and condensed tannins on rumen BH of both linoleic acid (LA) and α-LNA are still controversial (Buccioni et al., 2015; Minieri et al., 2014; Toral et al., 2011, Toral et al., 2013).

The aim of the present study was to evaluate the effects on milk yield and composition of moderate concentrations of CHT in the diet of grazing dairy ewes fed a concentrate containing extruded linseed. In particular, this study aimed to evaluate the effect of dietary CHT supplementation on concentrations of the urea, casein, and α-LNA and CLA in milk.

2. Material and methods

2.1. Animals

Ninety-six multiparous Sarda ewes, average body weight 44.2 ± 3.4 kg, at mid lactation (fourth month) were allotted to two groups each of 48 animals (control group, C group; experimental group, CHE group), which were balanced for age and parity. The handling of the animals was according to Institutional Animal Care and Use Committee (IACUC, 2004) of University of Florence. The ewes were milked daily at 07:00 and 18:00 h using a milking machine (43 kPa; 150 pulsation/min) and the daily milk yield was recorded.

2.2. Diets

Diets were formulated to meet requirements of a dairy ewe producing 1 kg of milk at 6.5% of fat according to Cannas et al. (2004). Animals of both groups grazed 8 h per day on the same pasture composed a mixture (1:1:1) of ryegrass (*Lolium multiflorum*), oat (*Avena sativa*) and white clover (*Trifolium repens*). The chemical and nutritional profile of pasture was: Dry matter (DM) 171.8 g/kg of fresh matter, crude protein (CP) 233.0 g/kg of DM, ether extract (EE) 37.1 g/kg of DM, neutral detergent fiber (assayed with heat stable amylase and expressed inclusive of residual ash, NDF) 371.2 g/kg of DM, acid detergent fire (ADF) 255.3 g/kg of DM, acid detergent lignin (ADL) 38.1 g/kg of DM, soluble protein (PS) 10 g/ kg of DM, non protein nitrogen (NPN) 51.0 g/kg of DM, Ash 140.3 g/kg of DM, non fiber carbohydrates (NFC) 218.4 g/kg of DM. The feeding regimen of the two groups differed only in the type of concentrate offered (data are expressed on DM basis): C group received 450 g DM/head per day of the control concentrate (C) containing 90 g of extruded linseed, whereas the other group (CHE group) received 500 g DM/head per day of the experimental concentrate (CHE) containing 90 g of extruded linseed and 40 g of a commercial CHT extract (Gruppo Mauro Saviola s.r.l., Radicofani, Siena, Italy). Chestnut tannin extract was previously characterized by Romani et al. (2013) and contained 750 g of equivalent tannic acid/kg DM, determined according to Burns (1963). Extruded linseed contained 350 g/kg DM of oil and 330 g/kg DM of crude protein. The ingredients and chemical composition of the concentrates are reported in Table 1. The concentrates were pelleted and molasses was added to both the concentrates to avoid the choice of dietary components by animals and to improve the palatability of the feeds. The amount of concentrates offered was established on the basis of the expected intake of pasture estimated by Cornell Net Carbohydrates and Protein System for sheep (Cannas et al., 2004) and taking into consideration the smaller concentration of crude protein and net energy of CHE compared to C, due to the inclusion of CHT.

2.3. Experimental design

Concentrates were offered during the morning (7:00) and the afternoon (18:00) milking and the daily intake of DM (DMI) was individually registered on the basis of residuals. The trial lasted 7 weeks, after a 3 weeks adaptation period. All the animals were weighed at the beginning and at the end of the experiment. Once a week, milk samples from each individual ewe were collected during both milkings and then combined in a single sample according to the morning and afternoon yield.

2.4. Proximate analysis of diets

Samples of pasture and concentrates were analyzed for proximate composition according to AOAC (1995) procedures (DM, 930.15; CP, 976.06; EE, 920.39; ash, 942.05) while the fiber fractions (NDF, ADF, ADL) were analysed after Goering and Van Soest (1970). NPN and PS were determined according to Licitra et al. (1996). NFC content was calculated according to NRC (2001). Net Energy lactation (NEl) was estimated according to Cannas et al. (2004).

2.5. Milk analysis

Milk samples were analyzed for fat according to Gerber and Gerber-Van Gulik (ISO, 1975); milk proteins, urea, total solids and lactose contents were determined by infrared analysis (Milkoscan