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Effect of increasing amounts of olive crude phenolic concentrate in the diet of dairy ewes on rumen liquor and milk fatty acid composition

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ABSTRACT

Agro-industrial by-products contain several secondary plant metabolites, such as polyphenols, tannins, saponins, and essential oils. The effects of these compounds on animal metabolism may vary significantly according to the dose, the chemical nature of the molecules, and the overall composition of the diet. In the Mediterranean area, the olive oil extraction is associated with 2 by-products: olive pomace and wastewater, both rich in polyphenols. In particular, wastewater may be further processed to obtain olive crude phenolic concentrate (OCPC). An experiment was carried out aiming to evaluate animal performance, milk fatty acid (FA) profile, diversity of rumen microbial population, and rumen liquor FA profile in dairy ewes fed diets containing extruded linseed (EL) and increasing doses of OCPC. Twenty-eight Comisana ewes in mid lactation were allotted to 4 experimental groups. The experiment lasted 5 wk after 3 wk of adaptation. Diets were characterized by lucerne hay administrated ad libitum and by 800 g/ewe and day of 4 experimental concentrates containing 22% of EL on dry matter and increasing dose of OCPC: 0 (L0), 0.6 (L0.6), 0.8 (L0.8), and 1.2 (L1.2) g of OCPC/kg of dry matter. Milk yield was daily recorded and milk composition was analyzed weekly. At the beginning and at the end of the experiment, samples of rumen liquor were collected to analyze FA profile, changes in rumen microbial population, and dimethylacetal (DMA) composition. The inclusion of OCPC did not affect milk yield and gross composition, whereas milk from L0.8 and L1.2 sheep contained higher concentrations of linoleic (+18%) and α-linolenic acid (+24%) and lower concentration of the rumen biohydrogenation intermediates. A similar pattern was observed for rumen liquor FA composition. No differences were found in the diversity of the rumen microbial population. Total amount of DMA did not differ among treatments, whereas significant differences were found in the concentration of individual DMA; in the diet with a higher amount of OCPC, DMA 13:0, 14:0, 15:0, and 18:0 increased, whereas DMA 16:0 decreased. Probably the presence of polyphenols in the diet induced a rearrangement of bacteria membrane phospholipids as a response to the rumen environment stimulus. Overall, the use of OCPC allowed a significant increase in the polyunsaturated FA content of milk, probably due to a perturbation of the rumen biohydrogenation process. Further studies are needed to understand the correlation between diet composition and the pattern of DMA in rumen liquor.

Key words: biohydrogenation, milk fatty acid, dimethylacetal, olive polyphenol

INTRODUCTION

The use of extruded linseed (**EL**) in the diet of dairy ruminants has been extensively studied as a feeding strategy to modify milk fatty acid (**FA**) composition (Chilliard et al., 2007; Mele et al., 2011). The addition of EL in the ruminant diet leads to an enrichment of milk and cheese with bioactive FA, such as PUFAn-3, CLA, and vaccenic acid (**VA**; Mele et al., 2011; Pintus et al., 2013). However, more than 85% of PUFA contained in EL are usually biohydrogenated (Buccioni et al., 2015b). As consequence, high amounts of dietary EL are needed to obtain milk with a FA profile rich in PUFA suitable for the production of cheese with a

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proven positive effect on human health (Mele et al., 2011; Pintus et al., 2013), causing an increase in feeding costs.

Recent studies showed that feeding polyphenolic compounds to ruminants resulted in a perturbation of PUFA rumen biohydrogenation (**RBH**) with no detrimental effects on milk yield and quality (Ferlay et al., 2010; Buccioni et al., 2015a). Moreover, in vitro studies suggested interference between dietary polyphenols and rumen microbiome profile (Pallara et al., 2014; Costa et al., 2017).

Several agro-industrial by-products contain considerable amounts of plant secondary metabolites, including different kind of polyphenols or other compounds such as saponins and essential oils, able to modulate RBH and, consequently, FA composition of ruminant-derived products (Vasta et al., 2008).

Olive oil extraction results in 2 main by-products: olive pomace and wastewater. More than 98% of total olive polyphenols accumulate in the wastewater during the oil extraction process. Olive polyphenols may be separated from the rest of the wastewater by applying an enzymatic treatment with depolymerizing enzymes, followed by a separation with membrane filters (0.2 μm) as described in Servili et al. (2011). After this treatment, an olive crude phenolic concentrate (**OCPC**) is obtained, with a polyphenol content 4 times higher than that of the wastewater.

Olive crude phenolic concentrate is mainly composed by oleuropein-aglycone di-aldehyde (**3,4-DHPEA-EDA**) and verbascoside, whereas hydroxytyrosol (**3,4-DHPEA**) and tyrosol (**p-HPEA**) are usually present at lower amounts (Servili et al., 2011).

A previous in vitro study suggested that polyphenols from olive pomace were probably able to interfere with RBH of dietary PUFA (Pallara et al., 2014). However, in dairy ewes, the effect of pure olive polyphenols on milk yield and composition is only scarcely studied (Casamassima et al., 2014). To investigate the response of microbes to changes in the rumen environment as affected by specific diet ingredients, recently dimethylacetal (**DMA**) analysis of the rumen liquor has been proposed (Alves et al., 2013). Dimethylacetals derive from plasmalogen lipids and seems to play an important role in the membrane fluidity of gram-negative bacteria. In particular, DMA profile has been proposed as potential marker of the response of rumen bacteria to the environmental condition (Katz and Keeney, 1964; Minato et al., 1988; Goldfine, 2010).

Thus, the effect of the inclusion of increasing amounts of OCPC, in combination with EL as a source of PUFA, in the diet of dairy ewes on milk traits and FA composition and on DMA and FA composition of rumen liquor was evaluated. Moreover, the effect of OCPC on overall rumen microbial community diversity was also investigated.

MATERIALS AND METHODS

Experimental Design

Twenty-eight pluriparous Comisana ewes at mid-late lactation (107 ± 9 d) and homogeneous for BW (68 ± 2.5 kg), kept at the Experimental Section of the Department of Agriculture, Food and Environmental Science, University of Perugia (Italy), were randomly allotted into 4 groups and confined in multiple pens (7 animals per pen).

The experimental trial lasted 5 wk after 3 wk of adaptation to the dietary regimen adopted for the present study. In the experimental period, the animals received 4 different diets based on chopped lucerne hay administrated ad libitum (particle size >4 cm of length), 100 g of rolled barley, and 800 g/animal and day of a concentrate containing EL as source of PUFA, plus different amounts of OCPC to obtain different concentration of total polyphenols: 0 (**L0**), 0.6 (**L0.6**), 0.8 (**L0.8**), or 1.2 (**L1.2**) g/kg of DM. The OCPC was obtained from the filtration of fresh vegetation wastewater, according to the procedure described by Servili et al. (2011). All the concentrate ingredients were incorporated into pellets using a pelleting machine (CMS-IEM, Colognola ai Coli, Verona, Italy). Pellet diameter was 5 mm and the pelleting temperature ranged between 35 and 40°C. Ingredients and chemical composition of the experimental diets were reported in Table 1.

Diets were formulated according to the nutritional requirements of a dairy ewe weighing 68 kg and producing 1 kg of milk at 6.5% fat (Cannas et al., 2004). Four hundred grams of experimental concentrates and 50 g of rolled barley were individually fed during each milking until their consumption was completed.

The ewes were milked twice daily (0730 and 1730 h) using a milking machine (43 kPa; 150 pulsations/min) and daily milk yield was individually recorded. The handling of the animals was in accordance with the Institutional Animal Care and Use Committee of the University of Perugia.

Sampling and Analysis

Feed Sampling and Analysis. Feed and orts samples were collected weekly, dried at 40°C, ground using a Cyclotec 1093 mill (PBI International, Milan, Italy) with a mesh size of 1 mm, and then stored at −80°C until analysis.

The content of CP and ether extract were determined according to the AOAC methods (976.06 and 920.39,