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Ability of 3 tanniferous forage legumes to modify quality of milk and Gruyère-type cheese

M. Girard,*† F. Dohme-Meier,* D. Wechsler,‡ D. Goy,‡ M. Kreuzer,† and G. Bee*[1]
*Institute for Livestock Sciences ILS, Agroscope, Tioleyre 4, 1725 Posieux, Switzerland
†Institute of Agricultural Science, ETH Zurich, Universitaetstrasse 2, 8092 Zurich, Switzerland
‡Institute for Food Sciences IFS, Agroscope, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

ABSTRACT

Condensed tannins (CT) may affect ruminal biohydrogenation of dietary polyunsaturated fatty acids. A feeding experiment was conducted with 24 Holstein cows to evaluate whether diets containing CT from different forage legumes can increase polyunsaturated fatty acids, especially n-3 fatty acid content in milk and cheese, without affecting negatively their physicochemical and sensorial properties. Cows were assigned to 4 treatment groups (n = 6) for 52 d, divided into 2 periods: a control period (CoP) and an experimental period (ExP). During the CoP, cows received a basal diet composed of hay, corn silage, ExtruLin (Trinova Handel & Marketing AG, Wangen, Switzerland), concentrate, and alfalfa (AF) in a ratio of 45:25:5:7:18. In the ExP, in 3 of the 4 groups AF was replaced by either sainfoin (SF; 19% CT in dry matter) or 1 of 2 cultivars of birdsfoot trefoil [Polom (BP), 3% CT; Bull (BB), 5% CT]. At the end of each period, milk was collected on 3 consecutive days and analyzed for milk gross composition and fatty acid profile and was processed to Gruyère-type cheese. A trained panel assessed the sensory quality of raw milk and cheese using discriminative and descriptive tests. This experimental design consisting of AF in both the CoP and ExP allowed us to quantify effects due to lactation stage and experimental diets. In both the CoP and ExP, dry matter intake and milk yield did not differ among treatment groups. From the CoP to the ExP, milk urea content was reduced by 23% with SF, remained unchanged with BP, and tended to increase with AF and BB. The odor of the raw BB milk was judged to be different from AF milk. With SF, switching from the CoP to the ExP resulted in a 17% increase of the 18:3n-3 proportion in milk and cheese lipids. In BP cheese, the increase was 3%, whereas it tended to decrease in BB cheese. Additionally, the 20:5n-3 and 22:5n-3 proportions tended to increase in SF cheese from the CoP to the ExP. Compared with the AF cheeses, cheeses from cows fed CT-containing legumes were judged harder and tended to be less adhesive to the palate. In addition, SF and BP cheeses had less rind. In conclusion, feeding SF compared with BB and BP increased the content of 18:3n-3 in the milk and the cheese without a negative effect on flavor of the cheese. Despite a similar CT content, the 2 birdsfoot trefoil cultivars had opposite effects on milk urea and 18:3n-3 deposition, suggesting that, besides the content, the chemical structure may have had an important effect on the CT efficacy.
Key words: condensed tannins, milk, cheese, quality

INTRODUCTION

The fatty acid profile of milk and dairy products is considered to be not ideal with respect to human health and being one reason for the decrease in milk consumption in some industrialized countries (Cavadini et al., 2000; Haug et al., 2007). The main concern is the high proportion of some SFA, known to increase blood levels of total cholesterol and low-density lipoproteins in humans who consume large amounts of these types of fatty acids (Williams, 2000; Mensink et al., 2003; Shingfield et al., 2008). The rather low proportion of unsaturated fatty acids, in particular PUFA, in milk fat occurs despite the high PUFA content of grass-based dairy diets. For instance, the study of Dohme-Meier and Bee (2012) showed that less than 25% of the PUFA ingested is secreted in the milk. The main cause for the low PUFA transfer rate is the biohydrogenation of dietary PUFA and MUFA by rumen microbes, mostly bacteria belonging to *Butyrivibrio* genus. In the rumen, the isomerase from *Butyrivibrio fibrisolvens* converts 18:2n-6 (linoleic acid) to 18:2 *cis*-9,*trans*-11 (rumenic acid) and then hydrogenates 18:2 *cis*-9,*trans*-11 to 18:1 *trans*-11 (vaccenic acid). Starting with a different step, 18:3n-3 (α-linolenic acid) is isomerized to 18:3 *cis*-9,*trans*-11,*cis*-15, then converted to 18:2 *trans*-11,*cis*-15, but then also hydrogenated to 18:1 *trans*-11

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[1]Corresponding author: giuseppe.bee@agroscope.admin.ch

(Kepler et al., 1966). In the last step of the fatty acid biohydrogenation process in the rumen, 18:1 *trans*-11 is hydrogenated by *Butyrivibrio proteoclasticum* to 18:0 (stearic acid) (Wallace et al., 2006). The disappearance of 18:3n-3 and 18:2n-6 in the rumen was, on average, 93 and 85%, respectively (Doreau and Ferlay, 1993). From a dietetic point of view, 18:3n-3 and 18:2n-6 are important in human nutrition because the human body cannot synthesize them and both are precursors of nutritionally important long-chain fatty acids of the n-3 and n-6 families. They are widely studied for their positive effects on human health, especially 20:5n-3 (eicosapentaenoic acid), 22:5n-3 (docosapentaenoic acid), and 22:6n-3 (docosahexaenoic acid) (Deckelbaum and Torrejon, 2012).

Nutritional strategies in dairy cows that reduce ruminal biohydrogenation of dietary PUFA and MUFA could be the key to improve milk quality with respect to human requirements. Several studies based on the use of condensed tannins (**CT**) showed a potential for CT to modulate in vitro and in vivo biohydrogenation in the rumen by changing rumen microbial population, sometimes affecting both growth and protease activity of the bacteria (Jones et al., 1994; Khiaosa-Ard et al., 2009; Vasta et al., 2010). Recently, Buccioni et al. (2015) observed in vivo that feeding quebracho tannins (1.6% of DMI) to ewes increased the relative abundance of *Butyrivibrio fibrisolvens* and decreased that of *Butyrivibrio proteoclasticum* compared with a tannin-free diet. Likewise, Vasta et al. (2009b) hypothesized that CT might indirectly regulate Δ^9 desaturase expression, an enzyme involved in the conversion of 18:0 to 18:1 *cis*-9 and 18:1 *trans*-11 to 18:2 *cis*-9,*trans*-11 in the muscle and in the mammary gland via a modulation of absorbed fatty acids and protein levels. The results of these 2 previous experiments are consistent with the beneficial effect of CT on fat quality of ruminant products (Turner et al., 2005), but some failed to show an effect (Aprianita et al., 2014). Many factors can influence the bioactivity of CT, such as the proportion of CT in the diet, the chemical structure of CT, the length of the period during which CT are fed, and the type of diet to which CT were added. For instance, Vasta et al. (2009a) found that CT included in concentrate were more efficient against biohydrogenation than when offered together with green herbage.

Preserving PUFA and MUFA, making dietary products more susceptible to oxidation, could also have negative consequences from a consumer's point of view. It is, therefore, important to ensure that the final product (milk or cheese) still has a pleasant taste and is free of off-flavors. Thus, CT feeding might create more intense flavors and even off-flavors, which would be more perceptible in products in which fat is concentrated. For instance, during storage, buttermilk from milk rich in unsaturated fatty acids was found to be more likely to oxidize than buttermilk from milk richer in SFA (Kristensen et al., 2004). Furthermore, oxidized flavors, such as fishy flavor, were positively correlated with increased proportions of 18:2n-6 and 18:3n-3 in the milk fat after 8 d of storage (Timmons et al., 2001). Finally, when the products are kept for a longer period, such as ripened cheese, the incidence of oxidation may be especially high. Consequently, the use of diets containing plants rich in bioactive compounds (CT included) have to be evaluated carefully as to whether they influence the organoleptic quality of dairy products (Martin et al., 2005).

The hypotheses tested in the present study were (1) that it is possible to elevate the proportions of n-6 and, especially, n-3 PUFA in milk and cheese lipids, (2) but that this adversely affects its odor and flavor, and (3) that the occurrence and levels of these effects depend on genotype (species, cultivar) of the CT-providing plants. For this purpose, 3 legume genotypes with known different CT content and likely different CT properties were grown and they were added to a PUFA n-3-enriched diet for dairy cows to promote differences in ruminal biohydrogenation.

MATERIALS AND METHODS

Experimental Legumes

Four legume forages were cultivated in Posieux, Switzerland (latitude: 46°46′N, longitude: 07°06′E; altitude: 650 m) in 2012. These were a non-CT control legume, alfalfa (*Medicago sativa* cultivar Sanditi; **AF**), sainfoin (*Onobrychis viciifolia* cultivar Perly; **SF**), and 2 birdsfoots trefoil cultivars [*Lotus corniculatus* cultivars Bull (**BB**) and Polom (**BP**)]. From the second harvest, wilted forages were dried in a rotary barrel (type 5.0, Kunz, Langnau, Switzerland). The drying process was a succession of short heating and cooling periods repeated 3 times. The heating and cooling source delivered temperatures of 700 and 82°C, respectively. This process lasted for a total of 240 s. Finally, dried forages were ground (<3 mm) and pressed into 2-cm cylindrical pellets.

Animals, Diet Composition, and Sampling

The experiment was conducted in accordance with the Swiss guidelines for animal welfare and approved by the Swiss cantonal veterinary office (approval number: 2012_48_FR).

The feeding experiment, which lasted 52 d, was conducted with 24 lactating Holstein cows. At the