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Milk fatty acid profile in cows fed red clover- or alfalfa-silage based diets differing in rumen-degradable protein supply

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ABSTRACT

Polyphenol oxidase in red clover (RC) silage reduces lipolysis and consequently protects its constituent fatty acids (FA) against biohydrogenation by ruminal microorganisms. Fatty acid biohydrogenation could be further inhibited by reducing the nitrogen (N) supply to ruminal bacteria. To compare the effects of RC and alfalfa (AL) silage fed in diets differing in rumen-degradable protein supply on the transfer efficiency of polyunsaturated FA from diet to milk, and on the resulting FA profile of milk fat, 8 multiparous Holstein dairy cows (72 ± 17 days in milk) were used in a replicated 4×4 Latin square design (21-day periods including 14 days of adaptation). Four treatments were compared in a 2×2 factorial arrangement with AL or RC fed in diets formulated to provide 100% (RDP-100) or 85% (RDP-85) of calculated rumen-degradable protein requirements. Rumen-degradable protein concentrations were adjusted by varying the supplies of ground vs. steam-flaked corn grains, and untreated vs. heat-treated soybean meals. Feeding RC, as compared with AL diets, decreased ruminal ammonia-N concentration as well as urea-N in plasma and milk. Intakes of *c*9*c*12 18:2 and *c*9*c*12*c*15 18:3 were lower for cows fed RC, but their secretion in milk and their apparent transfer efficiency from diet were greater as compared to cows fed AL. As a result, feeding RC increased the concentrations of *c*9*c*12 18:2 and *c*9*c*12*c*15 18:3, but decreased the proportions of *t*11 18:1, *c*9*t*11 18:2, and *t*11*c*15 18:2 in milk fat. These results are consistent with the lower effective ruminal disappearance of *c*9*c*12 18:2 and *c*9*c*12*c*15 18:3 in RC as determined by a 96 h in sacco incubation, and the lower abundance of ruminal bacteria capable of hydrolyzing dietary lipids and hydrogenating polyunsaturated FA (*Ruminococcus albus* and *Ruminococcus flavefaciens*) in the rumen content. The apparent transfer efficiency of *c*9*c*12 18:2 from diet to milk was further increased by feeding RDP-85 as compared with RDP-100 diet. Feeding RC decreased concentrations of linear odd-chain FA (11:0, 13:0, 15:0, 17:0, and *c*9 17:1) and increased proportions of branched-chain FA (iso13:0, iso14:0, iso15:0, iso16:0, iso17:0, anteiso15:0, and anteiso17:0) in milk fat as compared with AL. In conclusion, as compared with AL, cows fed RC produced milk with greater concentrations of major forage FA (*c*9*c*12 18:2 and *c*9*c*12*c*15 18:3) and lower proportions of intermediates in ruminal biohydrogenation of these FA. Variations in milk

Abbreviations: ADF-ADF, acid detergent fiber expressed inclusive of residual ash; AL, alfalfa; aNDF-NDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DM, dry matter; DMI, dry matter intake; ERD, effective ruminal disappearances; FA, fatty acids; N, nitrogen; PPO, polyphenol oxidase; RC, red clover; RDP, rumen degradable protein; VFA, volatile fatty acids.

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fat concentrations of odd- and branched-chain FA, which are synthesized in the rumen by various microbial populations, may be reflecting the effects of forage source on ruminal fermentation.

1. Introduction

Some botanical compounds found in feeds have the potential to modify rumen microbial populations or chemical structure of feed matrix and thus, the fatty acid (FA) profile of milk fat (Lourenço et al., 2008). An example of such compounds is polyphenol oxidase (PPO), an enzyme found in red clover (RC) silage. One of the reported effects of PPO is a reduction in ruminal biohydrogenation of polyunsaturated FA (Van Ranst et al., 2011). The mode of action of PPO remains uncertain (Lee, 2014). One proposed mechanism is through the encapsulation of plant lipids in a protein-phenol complex, which would reduce the availability of lipids to the action of lipolytic enzymes, thus reducing substrates for biohydrogenation (Lee et al., 2010; Van Ranst et al., 2011). Previous studies have demonstrated a reduction in apparent ruminal biohydrogenation, and a greater transfer efficiency of *c*9*c*12*c*15 18:3 from diet to milk fat, when RC was compared with grass silage (Vanhatalo et al., 2007; Halmemies-Beauchet-Filleau et al., 2014; Lejonklev et al., 2013) or white clover silage (Dewhurst et al., 2003a; b). However, to our knowledge, these comparisons have never been studied with legumes other than clover, such as alfalfa (AL).

Following a similar mode of action involving PPO, RC has been reported to reduce the availability of N in the rumen (Winters and Minchin, 2001). Moreover, the RC content in phenolic compounds such as biochanin A could also reduce rumen N availability due to their inhibiting effect on growth of hyper ammonia-producing bacteria responsible for amino acid deamination in the rumen (Flythe and Kagan, 2010; Flythe et al., 2013). These effects on the availability of dietary N to microbial populations could contribute to the reduction in ruminal biohydrogenation rate of unsaturated FA observed when RC is fed to ruminants. In support of this hypothesis, Gerson et al. (1983) observed a linear increase in the rate of in vitro lipolysis and biohydrogenation of unsaturated FA with increasing crude protein (CP) content of diets fed to rumen donor sheep from 4.5 to 15.4%. No further increase was observed over this upper level of dietary CP content. In order to maintain production performance, the availability of N in the rumen could also be lowered by applying heat treatments to protein supplements which reduce their degradability (Stern et al., 1985; Scott et al., 1991), while maintaining total metabolizable protein supplied to the animal.

This experiment was therefore designed to test the hypotheses that: i) feeding RC containing active PPO will reduce lipid hydrolysis and biohydrogenation of its constituent FA in the rumen; ii) lowering the availability of dietary N to ruminal microorganisms by feeding heat-treated protein supplements will also inhibit these reactions; and iii) the protective effect of PPO against RC protein degradation will further interact with varying ruminal degradability of protein supplements resulting in a greater apparent transfer efficiency of dietary polyunsaturated FA to milk fat when compared with AL.

2. Materials and methods

2.1. Feeding trial

Alfalfa (*Medicago sativa* L.) and RC (*Trifolium pratense* L.) silages used in this experiment were produced in two different fields at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada). Both legume forages were cut with a mower conditioner at the early flowering stage of development. After wilting at around 35% DM, AL and RC were harvested using a forage chopper, and stored as silage in plastic bag silos (AgBag, St-Nazianz, WI, USA).

The feeding trial was conducted at the same site in an air-conditioned tie-stall barn. Eight multiparous lactating Holstein cows (72 ± 17 days in milk) fitted with rumen cannulas were used in a replicated 4×4 Latin square design balanced for residual effects. Four dietary treatments were studied in a 2×2 factorial arrangement: diets based on either one of two forage legumes (AL or RC) formulated to meet 100 or 85% of rumen-degradable protein (RDP-100 or RDP-85) requirement according to NRC (2001). Rumen-degradable protein concentrations were adjusted by varying the supplies of ground vs. steam-flaked corn grains, and untreated vs. heat-treated soybean meals (Table 1). Dry matter (DM) concentrations of AL and RC silages were determined on a weekly basis by near-infrared analysis using a Foss XDS^{TM} Rapid Content Analyzer (Foss, Hillerød, Denmark) at Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Sainte-Anne-de-Bellevue, QC, Canada) and proportions of ingredients in the total mixed rations were adjusted accordingly on an as fed basis. Cows had continuous access to water and were fed total mixed rations twice daily at 07:00 and 19:00 h for *ad libitum* intake.

Feeding periods of each dietary treatment were 21 days in length. Cows were weighed at the beginning and the end of each period on 3 consecutive days. From day 15 to 21, the amounts of feed offered and refused were recorded and samples of rations and refusals were collected daily. Feed and refusals samples were stored at $-20\,^{\circ}C$ for further analyses.

Cows were milked in their stalls twice daily, at 07:00 and 17:00 h. Milk production was recorded and milk samples were collected from days 15 to 21 of each period. Milk samples were divided into two aliquots. The first set of aliquots was stored