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Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio

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ABSTRACT

Diets based on red clover silage (RCS) typically increase the concentration of polyunsaturated fatty acids (PUFA) in ruminant milk and meat compared with grass silages (GS), an effect that has been attributed to higher activity of polyphenol oxidase in red clover, promoting ruminal escape of dietary lipid. Four multiparous Finnish Ayrshire cows in mid lactation fitted with rumen cannulas were used in a 4 × 4 Latin Square design with 21-d experimental periods to evaluate the effects of incremental replacement of GS with RCS on ruminal lipid metabolism, using the omasal sampling technique in combination with Cr-EDTA, Yb acetate, and indigestible neutral detergent fiber as markers. Treatments comprised total mixed rations offered ad libitum containing 600 g of forage/kg of diet dry matter, with RCS replacing GS in a ratio of 0:100, 33:67, 67:33, and 100:0 on a dry matter basis. Silages contained a high proportion of lipid as nonesterified fatty acids (NEFA), with no difference between forage species (75 and 73% for GS and RCS, respectively). Substitution of GS with RCS had no influence on the intakes of NEFA, polar lipid, triacylglycerol, diacylglycerol, monoacylglycerol, or total fatty acids (FA), but altered the ingestion of specific FA. Replacing GS with RCS decreased linearly 18:3n-3 and increased linearly 18:2n-6 intakes. Changes in the proportion of RCS in the diet had no effect on the amounts or on the relative proportions of different lipid fractions at the omasum. On average, NEFA, polar lipid, triacylglycerol, diacylglycerol, and monoacylglycerol accounted for 80, 12, 4.4, 2.4, and 0.8% of total FA in omasal digesta, respectively. Replacement of GS with RCS increased linearly the amount of esterified and nonesterified 18:3n-3 at the omasum. Flows of *cis*-9 18:1 and 18:2n-6 were also increased linearly in response to RCS in the diet, whereas 3,7,11,15-tetramethyl-16:0 at the omasum was decreased. Replacing GS with RCS in the diet decreased linearly the lipolysis of dietary esterified lipids in the rumen from 85 to 70%. Effects on lipolysis due to forage species were also associated with linear decreases in apparent ruminal 18:3n-3 biohydrogenation from 93 to 85% and a trend toward lowered biohydrogenation of *cis*-9 18:1 and 18:2n-6 in the rumen. However, forage species had no effect on the flow of bound phenols formed as a consequence of polyphenol oxidase activity at the omasum. In conclusion, despite minimal differences in the extent of lipolysis in silo, lipid and constituent FA in RCS were less susceptible to ruminal lipolysis and biohydrogenation compared with GS.
Key words: red clover, grass, lipolysis, biohydrogenation

INTRODUCTION

Meat and milk from ruminants are characterized by relatively high proportions of SFA and low amounts of PUFA, due at least in part to extensive lipolysis and biohydrogenation of dietary unsaturated FA in the rumen (Dewhurst et al., 2006; Shingfield et al., 2010). Ruminant-derived foods are an important source of lipid in the human diet (Shingfield et al., 2008b) and, therefore, increasing interest exists in developing nutritional strategies for altering the composition of ruminant milk and meat to improve long-term human health. Evidence from clinical and biomedical studies suggest that increasing milk fat 18:3n-3 and decreasing 12:0, 14:0, and 16:0 FA concentrations may lower cardiovascular disease risk and insulin resistance in humans without requiring a change in consumer eating habits (WHO, 2003; Shingfield et al., 2008b).

It is well established that replacing grass silage (**GS**) with red clover (*Trifolium pratense* L.) silage increases 18:2n-6 and 18:3n-3 concentration in milk (Dewhurst et al., 2006; Vanhatalo et al., 2007; Moorby et al., 2009)

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because of higher escape of these PUFA from the rumen (Dewhurst et al., 2003; Lee et al., 2003; 2006). However, the mechanisms explaining the influence of forage species on ruminal metabolism and higher transfer of PUFA from the diet into milk are not well defined, although several mechanisms have been postulated (Lee et al., 2007, 2010).

Red clover has a higher polyphenol oxidase (**PPO**) activity compared with grasses (Van Ranst et al., 2011). Inherent differences in the activity of this enzyme have been suggested to contribute to the effects of forage species on ruminal biohydrogenation of unsaturated FA due to an inhibition of plant-mediated lipolysis arising from the formation of electrophilic quinones liberated during the action of PPO on endogenous phenols (Lee et al., 2004; Van Ranst et al., 2009b). Quinones readily react with cellular nucleophiles, resulting in the binding and complexing of phenols with specific functional groups of lipid in forages, which may result in the physical entrapment of forage lipid within protein-phenol matrices (Lee et al., 2010). The aim of the present study was to provide further insight into the influence of forage species on the transformations of dietary lipid in the rumen of lactating cows by examining the effects of incremental replacement of GS with RCS on the flow of bound phenols, esterified lipid fractions, and NEFA at the omasum. Four cows were used in a 4 × 4 Latin square design to test the hypothesis that the effects of RCS on milk fat composition are related to lower lipolysis and biohydrogenation of dietary unsaturated FA in the rumen as a consequence of inherent differences in PPO activity between forage species.

MATERIALS AND METHODS

Forage Management

Grass silage was prepared on June 12 to 13, 2007, from primary growths of 3- and 4-yr leys of mixed timothy (*Phleum pratense* L. 'Tuukka') and meadow fescue (*Festuca pratensis* Huds. 'Antti' and 'Ilmari'). Swards were established using a seed mixture (Boreal Plant Breeding Ltd., Jokioinen, Finland) comprising 54% timothy and 46% meadow fescue. On April 17, 2007, grass leys were fertilized per hectare with 96 kg of N, 4 kg of K, 11 kg of Ca, 4 kg of Mg, 11 kg of S, 74 g of B, and 4 g of Se. Red clover silage (**RCS**) was prepared on September 5, 2007, from secondary growths of single-year leys (cultivar Jokioinen; Boreal Plant Breeding Ltd.). Growth of red clover was not fertilized before cropping. Forages were grown in Jokioinen (60°49′N, 23°28′E), cut using a mower conditioner, and harvested at a DM content of 221 and 194 g/kg for grass and red clover, respectively, with a precision chop harvester, and ensiled in bunker silos with a formic acid-based additive (760 g of formic acid and 55 g of ammonium formate, AIV 2 Plus; Kemira Ltd., Helsinki, Finland) applied at a rate of 5 L/t of fresh herbage. Mean herbage yields were 3,600 and 2,300 kg of DM/ha for grass and red clover leys, respectively. Bunker silos were opened on January 28, 2008.

Animals, Experimental Design, and Experimental Diets

All experimental procedures were approved by the National Animal Ethics Committee (Hämeenlinna, Finland) in accordance with guidelines established by the European Community Council Directives 86/609/EEC.. Four multiparous Finnish Ayrshire cows (*Bos p. taurus*; 599 ± 26.0 kg of BW, 108 ± 5.8 DIM, and producing 30.0 ± 1.84 milk/d) fitted with rumen cannulas (100-mm i.d.; Bar Diamond, Inc., Parma, ID) were allocated at random to experimental diets according to a 4 × 4 Latin square design with 21-d periods. Experimental treatments consisted of TMR containing 600 g of forage/kg of diet DM with RCS replacing GS in the diet in the DM ratio (RCS:GS) of 0:100, 33:67, 67:33, and 100:0. Forages were supplemented with a standard concentrate formulated to meet or exceed ME and protein requirements of lactating cows producing 35 kg of ECM/d (MTT Agrifood Research Finland, 2006). Formulation of experimental diets is presented in Table 1. Diets were offered ad libitum as 4 equal meals at 0600, 0800, 1800, and 2000 h. Cows were housed in a dedicated metabolism unit equipped with individual tie-stalls with continuous access to water and milked twice daily at 0700 and 1645 h.

Measurements and Sampling

Individual cow intakes were recorded daily throughout the experiment, but only measurements for the last 7 d were used for statistical analysis. During this period, representative samples of silage and concentrates for chemical analysis were collected, composited, and stored at −20°C. Additional samples for lipid analysis were also collected and stored at −80°C. The chemical composition of feeds, including the correction of silage DM content for volatile losses was determined using standard procedures (Halmemies-Beauchet-Filleau et al., 2013). The concentration of indigestible NDF (**iNDF**) was determined in duplicate by incubating 0.5- to 1-g samples within polyester bags (60 × 120 mm, 17-μm pore size) in the rumen for 12 d (Ahvenjärvi et al., 2000). The OM content of the indigestible residue was determined by ashing at 600°C for 18 h.