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Effect of forage conservation method on ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio

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ABSTRACT

The effect of forage conservation method on ruminal lipid metabolism and microbial ecology was examined in 2 complementary experiments in cows. Treatments comprised fresh chopped grass, barn-dried hay, or untreated (UTS) or formic acid-treated silage (FAS) prepared from the same grass sward. Preparation of conserved forages coincided with the collection of samples from cows offered fresh grass. In the first experiment, 5 multiparous Finnish Ayrshire cows (229 d in milk) were used to compare the effects of feeding diets based on grass followed by hay during 2 consecutive 14-d periods separated by a 5-d transition during which extensively wilted grass was fed. In the second experiment, 5 multiparous Finnish Ayrshire cows (53 d in milk) were assigned to 1 of 2 blocks and allocated treatments according to a replicated 3 × 3 Latin square design with 14-d periods to compare the effects of hay, UTS, and FAS. Cows received 7 or 9 kg/d of the same concentrate in experiments 1 and 2, respectively. Conservation of grass by drying, but not ensiling, decreased forage fatty acid content primarily due to losses of 18:2n-6 and 18:3n-3. Compared with grass, feeding hay had no effect on dry matter intake (DMI), rumen pH, or fermentation characteristics, other than increasing ammonia content, but lowered whole-tract organic matter and fiber digestibility (experiment 1). Relative to hay, silage increased DMI, rumen volatile fatty acid (VFA) concentrations, and molar proportions of butyrate, and decreased molar acetate proportions (experiment 2). Compared with UTS, FAS increased DMI, had no effect on rumen ammonia or VFA concentrations, but tended to lower rumen pH and the molar ratio of lipogenic to glucogenic VFA. Conservation method had no substantial effect on ruminal or whole-tract digestibility coefficients. Compared with fresh grass and silages, hay decreased lipolysis and biohydrogenation (BH) of dietary unsaturates in the rumen, resulting in similar flows of 18:2n-6 and 18:3n-3, but lower amounts of *trans*-11 18:1 and Δ11,13 18:2 at the omasum. The extent of silage fermentation had minimal influence on ruminal lipid metabolism. Treatments were not associated with changes in the relative abundance of specific bacteria known to be capable of BH or rumen protozoal numbers. In conclusion, conservation method altered forage lipids, the extent of lipolysis and BH in the rumen, and the flow of fatty acids at the omasum, in the absence of substantial changes in ruminal *Butyrivibrio* populations.
Key words: *Butyrivibrio*, cow, forage conservation, ruminal biohydrogenation

INTRODUCTION

Ruminant meat and milk are characterized by high relative proportions of SFA and low proportions of PUFA due, at least in part, to extensive biohydrogenation of dietary unsaturated FA in the rumen (Dewhurst et al., 2006; Kim et al., 2009; Shingfield et al., 2012a). In humans, consumption of excessive amounts of SFA, 12:0, 14:0, and 16:0, in particular, is associated with an increase in cardiovascular disease risk and development of insulin resistance and dyslipidemia (WHO, 2003; Shingfield et al., 2008b). Milk and dairy products are typically a major source of medium-chain and total SFA in the Western diet (Hulshof et al., 1999; Kris-Etherton et al., 2000), generating considerable interest in lowering the amounts of medium-chain SFA, and increasing *cis*-9 18:1, 18:2n-6, conjugated linoleic acid (**CLA**), and 18:3n-3 in ruminant milk to improve long-term human health (refer to Shingfield et al., 2008b).

Concentrations of medium-chain SFA are known to be lower, and those of *cis*-9 18:1, *trans*-11 18:1, and

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cis-9,*trans*-11 CLA are higher in milk from pasture compared with hay or silage (Dewhurst et al., 2006; Elgersma et al., 2006; Chilliard et al., 2007), but the underlying mechanisms are not well defined. Even though wilting of grass is associated with extensive losses of unsaturated FA (Dewhurst and King, 1998; Boufaïed et al., 2003a; Shingfield et al., 2005), the concentrations of 18:2n-6 and 18:3n-3 in milk on grass hay-based diets are often similar to that from pasture (Dewhurst et al., 2006; Ferlay et al., 2006; Coppa et al., 2011). Differences in forage ensiling method have relatively minor effects on forage FA content, unless secondary fermentation in silo is extensive (Dewhurst and King, 1998; Boufaïed et al., 2003a; Shingfield et al., 2005), but milk from silage-based diets typically contain lower concentrations of PUFA compared with fresh grass or hay (Dewhurst et al., 2006; Chilliard et al., 2007). The efficiency of transfer of 18:3n-3 from the diet into milk is higher on hay- than grass silage-based diets (Shingfield et al., 2005), with the implication that forage conservation method influences the extent of ruminal escape of forage PUFA. Furthermore, recent studies have provided evidence that shifts in rumen microbial communities may, at least in part, contribute to dietary induced differences in ruminal lipid metabolism and outflow of FA (Huws et al., 2010; Shingfield et al., 2012b).

The aim of the present study was to examine the influence of conservation method on forage lipids, ruminal lipid metabolism, rumen microbial ecology, and flows of FA at the omasum in lactating cows fed diets based on fresh or conserved grasses prepared simultaneously from the same swards of timothy and meadow fescue. Measurements of plasma lipid concentrations, mammary lipogenesis, and milk fat composition will be reported in a companion paper [A. Halmemies-Beauchet-Filleau, P. Kairenius, S. Ahvenjärvi, V. Toivonen, P. Huhtanen, A. Vanhatalo, D. I. Givens (University of Reading, Reading, UK) , and K. J. Shingfield, unpublished data].

MATERIALS AND METHODS

Forage Management

All experimental forages were prepared from the same primary growths of 3-yr leys of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grown in Jokioinen, Finland (60°49′N, 23°28′E). On April 25, 2005, grass leys were fertilized with N and K at a rate of 96 and 4 kg/ha, respectively. Experiment 1 started on June 6, 2005. On each morning over a 7-d period (June 13 to 19, 2005) corresponding to d 8 to 14 of experiment 1, approximately 600 kg of grass was collected using a precision chop forage harvester and treated with a formic acid-based additive (0.5 L/t; AIV II Plus; Kemira Ltd., Helsinki, Finland) in an attempt to ensure that the composition of chopped grass resembled, as far as possible, parent herbage. Once collected, fresh chopped grass was transported immediately to a dedicated metabolism unit and stored at +4°C before feeding out to minimize oxidative deterioration. On June 15, 2005, corresponding to d 10 of the first experiment (Figure 1), an area of the same pasture used for zero grazing was cut using a mower-conditioner and conserved as hay or silage. Grass used for the production of hay was wilted extensively in the field over a 4-d period to a final DM content of 750 g/kg, baled, transported to a barn and left to dry further at ambient temperature for 5 d before being fed on d 20 of experiment 1. Grass assigned for the production of silage was cut using a mower-conditioner, wilted in the field for 3 to 4 h to a DM content of 235 g/kg, before being collected with a precision-chop harvester and ensiled in bunker silos. Differences in silage fermentation characteristics were achieved by ensiling grass directly with no additive (untreated, **UTS**) or using a formic acid-based additive (**FAS**; AIV II Plus) applied at a rate of 6 L/t of grass to restrict fermentation in silo. The chop length of fresh grass and grass silages averaged 3 to 4 cm. Dried hay was chopped (average chop length of 5 cm) immediately before feeding out.

Animals, Experimental Design, and Experimental Diets

All procedures involving animals were approved by the Animal Experiment Committee of MTT Agrifood Research Finland in accordance with the Use of Vertebrates for Scientific Purposes Act of 1985. Five multiparous lactating Finnish Ayrshire cows (605 ± 25.7 kg of BW and 229 ± 31.7 DIM) fitted with rumen cannulas (100-mm i.d.; Bar Diamond Inc., Parma, ID) were used in the first experiment. Another group of 5 rumen-fistulated Finnish Ayrshire cows (617 ± 25.8 kg of BW and 53 ± 5.3 DIM) were recruited for the second experiment. For both experiments, cows were housed in a dedicated metabolism unit fitted with individual stalls with continuous access to fresh water.

In experiment 1, the effects of feeding diets based on fresh chopped grass and barn dried hay were compared over 2 consecutive 14-d periods (June 6 to July 8, 2005). During the first period, cows were allowed to strip graze the same pasture used for conservation for 7 d before being transported to the metabolism unit and then zero grazed for 7 d. During the second 14-d period, cows received diets based on barn-dried hay. During the transition from fresh chopped formic acid-treated grass to grass hay, cows were offered extensively wilted grass