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Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio

A. Halmemies-Beauchet-Filleau,*† A. Vanhatalo,† V. Toivonen,* T. Heikkilä,* M. R. F. Lee,‡[1] and K. J. Shingfield*[2]
*MTT Agrifood Research Finland, Animal Production Research, FI-31600 Jokioinen, Finland
†University of Helsinki, Department of Agricultural Sciences, PO Box 28, FI-00014 University of Helsinki, Finland
‡Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, United Kingdom

ABSTRACT

Diets based on red clover silage (RCS) typically increase the concentration of polyunsaturated fatty acids (PUFA) in ruminant meat and milk and lower the efficiency of N utilization compared with grass silages (GS). Four multiparous Finnish Ayrshire cows (108 d postpartum) fitted with rumen cannulas were used in a 4 × 4 Latin square design with 21-d periods to evaluate the effect of incremental replacement of GS with RCS on milk production, nutrient digestion, whole-body N metabolism, and milk fatty acid composition. Treatments comprised total mixed rations offered ad libitum, containing 600 g of forage/kg of diet dry matter (DM), with RCS replacing GS in ratios of 0:100, 33:67, 67:33, and 100:0 on a DM basis. Intake of DM and milk yield tended to be higher when RCS and GS were offered as a mixture than when fed alone. Forage species had no influence on the concentration or secretion of total milk fat, whereas replacing GS with RCS tended to decrease milk protein concentration and yield. Substitution of GS with RCS decreased linearly whole-tract apparent organic matter, fiber, and N digestion. Forage species had no effect on total nonammonia N at the omasum, whereas the flow of most AA at the omasum was higher for diets based on a mixture of forages. Replacing GS with RCS progressively lowered protein degradation in the rumen, increased linearly ruminal escape of dietary protein, and decreased linearly microbial protein synthesis. Incremental inclusion of RCS in the diet tended to lower whole-body N balance, increased linearly the proportion of dietary N excreted in feces and urine, and decreased linearly the utilization of dietary N for milk protein synthesis. Furthermore, replacing GS with RCS decreased linearly milk fat 4:0 to 8:0, 14:0, and 16:0 concentrations and increased linearly 18:2n-6 and 18:3n-3 concentrations, in the absence of changes in *cis*-9 18:1, *cis*-9,*trans*-11 18:2, or total *trans* fatty acid concentration. Inclusion of RCS in the diet progressively increased the apparent transfer of 18-carbon PUFA from the diet into milk, but had no effect on the amount of 18:2n-6 or 18:3n-3 at the omasum recovered in milk. In conclusion, forage species modified ruminal N metabolism, the flow of AA at the omasum, and whole-body N partitioning. A lower efficiency of N utilization for milk protein synthesis with RCS relative to GS was associated with decreased availability of AA for absorption, with some evidence of an imbalance in the supply of AA relative to requirements. Higher enrichment of PUFA in milk for diets based on RCS was related to an increased supply for absorption, with no indication that forage species substantially altered PUFA bioavailability.

Key words: red clover, grass, milk fatty acid, nitrogen metabolism

INTRODUCTION

Red clover (*Trifolium pratense*) is a common forage legume grown in northern Europe, the United States, and Canada. Forage legumes form an integral part of organic and low-input milk-production systems, owing to the ability to capture atmospheric N_2, lowering the necessity for additional N. Given the rising costs of inorganic N fertilizers and attempts to decrease the environmental footprint of ruminant livestock production, red clover may also be advantageous in conventional milk-production systems. Compared with grasses, red clover has a high intake and milk-production potential (Dewhurst et al., 2003b; Moorby et al., 2009; Steinshamn, 2010), but the efficiency of dietary N utilization for milk protein synthesis is often lower (Dewhurst et al., 2003b; Moorby et al., 2009; Vanhatalo et al., 2009). It has been postulated that either an excessive or an

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[1]Current address: School of Veterinary Science, University of Bristol, Langford, Somerset, BS40 5DU, United Kingdom.
[2]Corresponding author: kevin.shingfield@mtt.fi

imbalanced supply of AA available to the mammary glands, or possibly both, may limit the conversion efficiency of dietary N into milk with red clover silage (**RCS**) diets, with a potential deficiency of Met being implicated (Lee et al., 2009; Vanhatalo et al., 2009).

Meat and milk from ruminants are characterized by high concentrations of SFA and a low abundance of PUFA, due at least in part, to extensive biohydrogenation of dietary unsaturated FA in the rumen (Dewhurst et al., 2003a; Shingfield et al., 2013). Ruminant-derived foods are an important source of lipid in the human diet (Shingfield et al., 2013) and, therefore, considerable interest exists in developing nutritional strategies to lower 12:0, 14:0, and 16:0 and increase 18:3n-3 in ruminant milk fat with potential to improve long-term human health (Gebauer et al., 2006; Shingfield et al., 2008). It is well established that milk from diets based on RCS contains much higher 18:2n-6 and 18:3n-3 concentrations compared with ensiled grass (Dewhurst et al., 2006; Vanhatalo et al., 2007; Moorby et al., 2009). At least part of the enrichment in milk can be attributed to higher amounts of 18:2n-6 and 18:3n-3 escaping the rumen (Dewhurst et al., 2003a,b), but the influence of forage species on the digestion, absorption, and utilization of FA for milk fat synthesis in lactating cows has not been extensively investigated.

Red clover has a higher polyphenol oxidase (**PPO**) activity compared with grasses, differences that have been suggested to contribute to the effects of forage species on proteolysis and lipolysis in silo and in vivo (Lee et al., 2004; Merry et al., 2006; Van Ranst et al., 2011). Previous investigations comparing RCS and grass silage (**GS**) diets have characterized the effects of forage species on ruminal N and lipid metabolism and milk FA composition in lactating cows under conditions typical for organic or low-input milk production (Dewhurst et al., 2003a,b; Moorby et al., 2009).

The aim of the present study was to provide further insight into the influence of forage species on the transformations of dietary protein in the rumen, efficiency of N utilization, and bioavailability of FA in cows fed diets relevant to more intensive milk-production systems. Four cows in a 4 × 4 Latin square design were used to test the hypotheses that the effects of RCS on the efficiency of N utilization and milk FA composition are related to changes in the amount and bioavailability of AA and FA available for absorption.

MATERIALS AND METHODS

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 (599 ± 26.0 kg of BW, 108 ± 5.8 DIM, and producing 30.0 ± 1.84 kg of 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 with 21-d periods. A detailed description of experimental treatments, management of grass and red clover leys, and preparation of experimental silages are provided in a companion paper (Halmemies-Beauchet-Filleau et al., 2013c). In brief, experimental treatments comprised TMR containing 600 g of forage/kg of diet DM with RCS replacing GS in the diet in the DM ratio of 0:100, 33:67, 67:33, and 100:0 (treatments **GS**, **GRC**, **RCG**, and **RCS**, respectively). Silages were prepared from mixed timothy (*Phleum pratense* L. 'Tuukka') and meadow fescue (*Festuca pratensis* Huds. 'Antti' and 'Ilmari') and red clover (*Trifolium pratense* L. 'Jokioinen') swards treated with 5 L/t of formic acid-based additive (760 g of formic acid and 55 g of ammonium formate, AIV 2 Plus; Kemira Ltd., Helsinki, Finland). Forages were supplemented with a standard concentrate (Table 1). Diets were offered ad libitum as 4 equal meals at 0600, 0800, 1800, and 2000 h. The chemical composition of individual feed ingredients and experimental diets is shown in Table 1. Cows were housed in a dedicated metabolism unit fitted with individual tie-stalls with continuous access to water. Cows were milked twice daily at 0700 and 1645 h. At the end of the experiment, cows weighed 603 ± 29.3 kg.

Measurements and Sampling

Individual cow intakes were recorded throughout the experiment, but only measurements for the last 7 d of each period were used for statistical analysis. During this time, representative samples of silage and concentrates were collected, composited, and stored at −20°C before chemical analysis. The chemical composition of feeds, including the correction of silage DM content for volatile losses was determined using standard methods (Halmemies-Beauchet-Filleau et al., 2013a). Starch in feeds was analyzed according to Salo and Salmi (1968). The concentration of indigestible NDF (**iNDF**) in silages and concentrates 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. Body condition scores were recorded on a scale of 1 (thin) to 5 (fat) at the start of the experiment and at the end of each experimental period.