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Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows

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ABSTRACT

Twenty-four multiparous Holstein-Friesian dairy cows were used in a replicated 4 × 4 Latin square changeover design experiment to test the effects of changing from ryegrass (*Lolium perenne*) silage to red clover (*Trifolium pratense*) silage in graded proportions on feed intakes, milk production, milk organoleptic qualities, and whole-body nitrogen partitioning. Four dietary treatments, comprising ad libitum access to 1 of 4 forage mixtures plus a standard allowance of 4 kg/d dairy concentrates, were offered. The 4 forage mixtures were, on a dry matter (DM) basis: 1) 100% grass silage, 2) 66% grass silage: 34% red clover silage, 3) 34% grass silage: 66% red clover silage, and 4) 100% red clover silage. In each of 4 experimental periods, there were 21 d for adaptation to diets and 7 d for measurements. There was an increase in both DM intakes and milk yields as the proportion of red clover in the diet increased. However, the increase in milk yield was not as great as the increase in DM intake, so that the efficiency of milk production, in terms of yield (kg) of milk per kg of DM intake, decreased. The concentrations of protein, milk fat, and the shorter chain saturated fatty acids decreased, whereas C18 polyunsaturated fatty acids (PUFA) and long-chain PUFA (C20+) increased as the proportion of red clover in the diet increased. There was little effect of dietary treatment on the organoleptic qualities of milk as assessed by taste panel analysis. There were no effects on the aroma of milk, on aftertaste, or overall liking of the milk. Milk was thicker and creamier in color when cows were fed grass silage compared with red clover silage. The flavor of milk was largely unaffected by dietary treatment. In conclusion, increasing the proportion of red clover in the diet of dairy cows increased feed intakes and milk yields, decreased the concentration of fat and protein in milk, increased PUFA for healthiness, and had little effect on milk organoleptic characteristics.

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INTRODUCTION

As a legume, red clover (*Trifolium pratense*) requires no nitrogen fertilizer making it suitable as a home-grown protein source for low-input or organic farming systems (Wilkins and Jones, 2000). Accumulation of soil N following a crop of red clover has the potential to reduce fertilizer N requirements (Lafond and Pageau, 2007) and thus reduce greenhouse-gas emissions from fertilizer use when included as part of a rotation. The potential use of ensiled red clover for milk production has been recognized for many years, with early data suggesting that feed intakes are greater than with grass silage (Castle and Watson, 1974). More-recent work has demonstrated improvements in milk production when feeding red clover as the sole forage compared with grass silage (Bertilsson and Murphy, 2003; Dewhurst et al., 2003b; Al-Mabruk et al., 2004), primarily because of greater intakes of red clover silage than grass silage. However, N digestibility of red clover silage tends to be lower than that of grass silage (Bertilsson and Murphy, 2003; Dewhurst et al., 2003b) and the efficiency of use of feed N tends to be lower as more N is excreted in feces of cows consuming red clover silage.

Although grass pastures can be both grazed and ensiled in a single season, the opportunity for use of this system of management is much less for red clover. The area of land that a dairy farmer can devote to a red clover crop for ensiling is likely to be small; therefore, red clover silage is most likely to be used strategically at a particular stage of lactation or mixed with other crops. Thomas et al. (1985) found immediate advantages but no beneficial carry-over effects of feeding red clover silage alone in early lactation or later in the same lactation, although mixing red clover with grass silage can offer benefits similar to those of feeding red clover silage alone (Bertilsson and Murphy, 2003; Dewhurst et al., 2003b). However, there is some evidence that the organoleptic qualities of milk produced using red clover silage are not as good as those pro-

duced from grass silage (Bertilsson and Murphy, 2003). This is potentially a result of changes in the fatty acid profile of milk produced from red clover silage, with increased concentrations of polyunsaturated fatty acids (**PUFA**) (Dewhurst et al., 2003b; Al-Mabruk et al., 2004; Vanhatalo et al., 2007), which may also lead to a reduction in the keeping qualities of the milk produced (Al-Mabruk et al., 2004). Increased concentrations of PUFA in milk result from the activity of the enzyme polyphenol oxidase (**PPO**; EC 1.10.3.1), which is present in high concentrations in red clover and reduces lipolysis and hence microbial biohydrogenation of fatty acids in the rumen (Lee et al., 2007, 2008).

Escherichia coli O157 is pathogenic in humans, with dairy herds being a potential source of contamination of the food chain (Wells et al., 1991; Zhao et al., 1995). Certain plant secondary compounds, including several coumarins, are known to inhibit the growth of *E. coli* O157 when incubated in rumen fluid (Duncan et al., 1998). Red clover contains high concentrations of plant secondary compounds, and although it is best known for its isoflavanoid content, red clover hay has been shown to reduce the amount of *E. coli* in cattle rumen contents (Jacobson et al., 2002). Feeding different conserved forages also had effects on fecal bacteria counts (Jacobson et al., 2002), and it was hypothesized that changing red clover silage to grass silage may alter the fecal bacterial population, including *E. coli* O157 counts.

The objectives of this experiment were to investigate the effect of mixing red clover and ryegrass silages at different ratios on feed intake, milk production, and whole-body N partitioning in dairy cows. The effects of diet on milk fatty acid profiles and the organoleptic qualities of the milk produced were also investigated. Diet effects on gut microbial populations were also investigated by studying feces.

MATERIALS AND METHODS

Cows and Management

All procedures used in this experiment were licensed and regulated by the UK Home Office under the Animals (Scientific Procedures) Act of 1986. Twenty-four multiparous Holstein-Friesian dairy cows were used in the experiment, with a mean BW of 651 (SD 56.0) kg and 103 (SD 28.7) DIM. The experimental design was a 4 × 4 Latin square with 6 replicated squares. Cows were kept in a free-stall barn at all times, except for a subset of the animals (n = 8) that were transferred to a metabolism unit for feed digestibility and nitrogen partitioning measurements for 10 d during each period of the experiment. Each experimental period lasted 28 d and was divided into an adaptation period (the first 21 d) and a measurement period (the last 7 d).

For 3 wk before the start of the experiment all animals were fed a common covariate diet with ad libitum access (with refusals of at least 0.1 of the quantity offered) to grass silage and red clover (1:1 mix on a fresh matter basis) plus 4 kg/d of a standard purchased dairy concentrate feed (Dairy XGM 18 Nuts, Welsh Feed Producers, Carmarthen, UK). Cows were assigned to 1 of 6 replicate groups (Latin squares) of 4 animals shortly before the start of the experiment based on milk yield data collected over the week before the start of the experiment. Animals were ranked from highest to lowest milk yields: the top 4 yielding cows were then allocated to square 1, the next 4 yielders to square 2, and so on, with the lowest 4 yielding animals allocated to square 6. Within each Latin square, animals were allocated at random to each of the 4 treatments. Cows in Latin squares 1 and 4 were used for digestibility and whole-body N partitioning measurements.

Four dietary treatments were used in the experiment. These consisted of ad libitum access to 1 of 4 forages, comprising mixtures of grass and red clover silages, together with an allowance of 4 kg of the same concentrate per day (offered as 2 kg at each milking, at approximately 0500 and 1500 h). The forage treatments, prepared on a DM basis, were 1) 100% perennial ryegrass silage (*Lolium perenne*), **R0**; 2) 66%:34% perennial ryegrass:red clover silage, **R34**; 3) 34%:66% perennial ryegrass:red clover silage, **R66**; and 4) 100% red clover silage, **R100**. The grass silage was prepared as a large bunker from a perennial ryegrass sward using an inoculant (Powerstart, Genus plc, Nantwich, UK) according to the manufacturer's guidelines. The grass was chopped at collection before ensilage, with the forage harvester knives set to give a chop length of approximately 25 mm. The red clover silage was prepared as large round bales from second and third cuts of a monoculture sward of red clover (cv. 'Milvus'), also using Powerstart as an inoculant at ensiling; the red clover crop was not chopped before baling. During the experiment, the red clover used was a mixture of the 2 silage cuts in a ratio of 1 bale second-cut to 1 bale third-cut. The concentrate feed, that was fed in equal quantities to all cows, comprised wheat (40%), extracted rapeseed meal (17.5%), palm kernel expellers (10%), citrus pulp (7.5%), high-protein soy (6.5%), molasses (6.0%), extracted sunflower meal (6.0%), Megalac (3.0%; Volac International Ltd., Royston, UK), sugar beet pulp (1.5%), and a mineral and vitamin mix (2.0%) certified to contain 9,000 IU of vitamin A, 2,000 IU of vitamin D3, 30 mg of α-tocopherol, 50 mg of copper (as sulfate, carbonate, and metalosate), and 750 μg of selenium (as sodium selenite) per kilogram of the fresh concentrate.

Grass and red clover silages and the 2 silage mixes were prepared by thorough mixing in a Storti Labrador