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Effect of feeding fresh or conditioned red clover on milk fatty acids and nitrogen utilization in lactating dairy cows

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ABSTRACT

Polyphenol oxidase (PPO) in conditioned red clover (ensiled or cut and crushed) reduces both proteolysis and lipolysis in the herbage, which has led to increases in N use efficiency and polyunsaturated fatty acid (PUFA) content of milk when offered to dairy cows. In damaged plant cells, PPO is activated and binds protein through the formation of protein-bound phenols. This study investigated a) whether freshly cut red clover could increase N use efficiency and milk PUFA concentrations in dairy cows or whether PPO enzymes require prior activation before feeding to elicit a response, and b) apparent whole-tract amino acid digestibility to help determine the effect of PPO on amino acid utilization. Six multiparous Holstein × Friesian dairy cows in mid-lactation were allocated at random to 1 of 3 dietary treatments in a 3 × 3 Latin square: a control treatment of grass (low PPO, G); red clover (high PPO, RC), and conditioned red clover (high fully activated PPO, CRC). The CRC herbage was cut and chopped in the field and then transported with the G and RC herbages to the animal house. Each period consisted of a 2-wk adaptation to diet and a week of measuring dietary effects (N balance and milk collection). The PPO activity was greatest in the RC treatment as fed, whereas activation of latent PPO enzyme and protein-bound phenol levels were greatest in the CRC diet. Dry matter and total fatty acid intakes were comparable across treatments (18.8 kg/d and 550 g/d, respectively). Milk yields and total fatty acid content were similar across treatments (32.6 kg/d and 34.8 mg/mL, respectively). Cows offered either RC or CRC had greater levels of protein, C18 PUFA and total long-chain PUFA in their milk than animals offered grass with no difference between RC and CRC. Nitrogen intakes, and output in milk, urine, and feces were greater in cows offered the 2 red clover treatments than G, with no difference between RC and CRC. However, there were no differences in N use efficiency among diets as measured by the proportion of feed N converted into milk N, possibly as the result of the excessive supply of N with the red clover diets. Amino acid apparent whole-tract digestibilities were greater when on RC than G diets and intermediate when on CRC for all amino acids, with the exception of Met, which was reduced in cows on both red clover diets compared with G. It is proposed that the PPO trait could show more benefit to ruminants if red clover was fed in combination with lesser N-containing forages or if red clover was bred to contain less N.
Key words: red clover, polyphenol oxidase, N use efficiency, polyunsaturated fatty acid

INTRODUCTION

The rate of proteolysis of forage proteins in the rumen is a key determinant of the efficiency of conversion of feed N into products or pollutants. The efficiency of conversion of feed N into milk (20 to 30%) or meat (10 to 20%) by ruminants is often well below potential (>40%), and is particularly minimal with diets based on poor quality grass silages (Dewhurst et al., 1996) or grazed herbage (Beever et al., 1986). At the same time, the biohydrogenation of fatty acids in the rumen following lipolysis of plant glycerol-based lipids reduces the polyunsaturated fatty acid (**PUFA**) to saturated fatty acid (**SFA**) ratio of both meat and milk. The feeding of red clover silage has been shown to reduce both N and PUFA losses when offered to dairy cows (Dewhurst et al., 2003). This is thought to be related to the enzyme polyphenol oxidase (**PPO**), which is associated with the browning losses in fruit and vegetables but has also been associated with reductions in both proteolysis and lipolysis both in the silo (Albrecht and Muck, 1991; Lee et al., 2008a) and the rumen (Albrecht and Broderick, 1992; Lee et al., 2007). This protection may be related to the formation of quinones produced by the PPO-catalyzed oxidation of vacuolar diphenols. The exact mechanism of the subsequent quinone-protein reaction is not fully understood. The amine group of free AA

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reacts with quinones forming phenols that are susceptible to further oxidation reactions (Rouet-Mayer et al., 1993). As peptides, certain side chains, namely amines (e.g., Lys, Rouet-Mayer et al., 1993; Akagawa et al., 2005) and sulfo (e.g., Met, Igarashi and Yasui, 1985; Rouet-Mayer et al., 1993), have been highlighted as potential targets of quinone attack, which, once bound, reform phenols covalently bound to the protein. The resultant phenol-bound protein complex reduces proteolysis and lipolysis in the silo through deactivation of plant proteases (Jones et al., 1995) and lipases (Lee et al., 2004). However, in the rumen plant enzymes are less active than the numerically superior microbial enzymes and subsequently proteolysis appears to be reduced due to the PPO-induced complexing of leaf proteins, reducing digestibility (Winters and Minchin, 2001). Lipolysis in the rumen is also reduced through the actions of PPO before ingestion (Lee et al., 2007), possibly as a consequence of entrapment of lipid micelles in the phenol-bound protein complex as postulated by Lee et al. (2008b).

Although the effect of PPO in red clover silage has been established, its role in grazed red clover is yet to be determined. Red clover PPO enzyme exists within the plant in both the active (approximately 10%) and latent (approximately 90%) forms; the former is active within the cell at neutral pH, whereas the latter requires activation (Lee et al., 2008c). Conversion of the latent to the active form (activation) can occur in red clover plant tissue either through proteolytic activity or mixing with endogenous phenolic substrate (phaselic acid and clovamide; Winters et al., 2008). Activation is prevented in healthy tissue due to the compartmentalization of the enzyme (chloroplast) and substrate (vacuole). Therefore, for PPO activation and activity to occur, cell damage (mixing of enzyme and substrate and activating latent PPO) and aeration (supply of oxygen for the oxidation reaction) are required. These 2 criteria are easily met during silage making, but during grazing the anaerobic nature of the rumen provides only a small window of opportunity during mastication (cell damage and aeration) for PPO activation (Lee et al., 2008c).

The objectives of this study were to determine whether offering conditioned red clover versus freshly cut red clover, with grass used as a negative control, would result in an elevation in N use efficiency and milk PUFA content as a consequence of PPO activation. In addition, apparent whole-tract AA digestibilities were assessed to indicate the potential effects of phenol covalent binding to AA induced through PPO activity on their subsequent digestibility.

MATERIALS AND METHODS

Experimental Design

The experiment involved 6 multiparous Holstein × Friesian dairy cows in midlactation on 3 dietary treatments in a changeover-design experiment (3 × 3 Latin square): 1) grass as the control (**G**), low PPO; 2) red clover (**RC**), high PPO; and 3) conditioned (chopped, bruised) red clover (**CRC**), high PPO and activated latent PPO. Measurements included milk fatty acid profiles, N partitioning into milk, urine, and feces with forage intake, and apparent whole tract AA digestibility and fecal output.

Diets and Allocation to Treatment

Italian ryegrass (*Lolium multiflorum*) and red clover (*Trifolium pratense*) were sown in plots with approximate areas of 0.7 and 1.5 ha, respectively. Six multiparous Holstein × Friesian dairy cows (650 ± 34.8 kg) in midlactation (with similar milk yields and stage of pregnancy) were maintained on a grass silage diet before allocation to treatment and during a 1-wk covariate period. Mean milk yields were recorded during the covariate, and the 3 cows with the 3 greatest mean yields were allocated within a "high-yielding group" (cows 1 through 3), and the cows with the 3 least yields allocated within a "low-yielding group" (cows 4 through 6). Within each Latin square, the cows from each group were allocated at random to 1 of 3 treatments listed above (Cochran and Cox, 1957). Each period consisted of a 2-wk adaptation followed by a 1-wk measurement period.

Feeding

Four weeks before the commencement of the study, each forage plot was split into 4 subplots. Each of these 4 subplots was subsequently managed to provide sufficient forage at 4 weeks of regrowth to be zero grazed (cut and carried) for each week of the experiment. Fresh G and RC were harvested daily in the early afternoon using a plot harvester (Haldrop 1500, J. Haldrop, Løgstør, Denmark). The CRC treatment was cut using a circular mower and chopper and was left as a swath in the field for about 1 h before collection. All forages were cut to a height of 5 cm above soil level. The cut forages (about 350 kg of fresh matter for each treatment per day) were then transported from the field, and 80 kg was placed into each of 12 large weld mesh containers (120 × 80 × 100 cm, length × depth × height), to give 4 baskets of each treatment. The CRC treatment was

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