



Milk production and methane emissions from dairy cows fed a low or high proportion of red clover silage and an incremental level of rapeseed expeller



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ABSTRACT

This study evaluated the effects of including increasing levels of rapeseed expeller in dairy cow diets with a low or high proportion of red clover silage on milk production and methane emissions. A total of 32 lactating Swedish Red dairy cows were used in a cyclic change-over design with three periods of 21 days, in a 2×4 factorial arrangement of treatments. The total mixed ration consisted of 600 g/kg dry matter (DM) of forage and 400 g/kg DM of concentrate on a DM basis. The forage treatments consisted of a 30:70 or 70:30 ratio of grass to red clover silage (RC30 and RC70). A basal supplement consisted of crimped barley and premix, formulated to contain 130 g CP/kg DM. For the three additional concentrate supplements, crimped barley was gradually replaced with incremental levels of rapeseed expeller to reach 170, 210 or 250 g CP/kg DM. No differences in feed intake were found between RC30 and RC70, but a positive response was found to increased dietary CP concentration from rapeseed expeller. Increasing proportion of red clover silage did not have any effect on production, while increasing dietary CP concentration increased yield of milk, energy corrected milk (ECM) and milk protein. Nitrogen efficiency was higher with diet RC30 than with RC70 and decreased with increasing dietary CP concentration, while milk urea nitrogen increased. Methane (CH₄) emissions per unit feed intake decreased with dietary CP concentration and tended to increase with increasing proportion of red clover silage in the diet. Increased CP intake from red clover silage in the diet of dairy cows had no positive effect on CH₄ emissions.

1. Introduction

Red clover (*Trifolium pratense* L) is the most common forage legume grown at northern latitudes in Europe, where grass or grass-red clover silage is the main ingredient in dairy cow diets. Due to its ability to fix atmospheric nitrogen (N), inclusion of red clover is of high value in leys in organic and low input dairy production systems. Future interest in using red clover within conventional dairy production may also increase with the rising cost of N fertiliser. When leys are composed of both grass and red clover, the regrowth has a higher proportion of red clover and a different quality of the grass, than the primary growth. On-farm, this results in forages of different qualities owing to the differences in feed characteristics between red clover and grasses.

Red clover silage fed as a sole forage and in mixtures with grass silage has been reported to have better potential to increase dry matter intake (DMI) and milk yield than diets containing pure grass silage in the forage ration (Dewhurst et al., 2003b; Moorby et al., 2009). When revising their relative silage DMI (SDMI) index, Huhtanen et al. (2007)

showed that when dairy cows were fed mixtures of grass and legumes, SDMI was higher than predicted from the mean of grass and legumes fed alone.

Inclusion of rapeseed feedstuffs have increased milk production compared with soybean meal when fed as a protein supplement to dairy cows on a grass silage-based diet (Shingfield et al., 2003; Gidlund et al., 2015). Moreover, Martineau et al. (2013) found that feeding rapeseed feedstuffs increased the uptake of essential amino acids in the small intestine compared with soybean meal and other protein supplements. Furthermore, addition of rapeseed feedstuffs is reported to increase the omasal flow of non-ammonia N (NAN), with the positive production responses to the protein supplement being attributed to increased dietary NAN flow to the omasum (Ahvenjärvi et al., 1999).

Red clover silage increases the flow of dietary and total NAN from the rumen compared with grass silage (Dewhurst et al., 2003a; Vanhatalo et al., 2009). The enzyme polyphenol oxidase (PPO) has been cited as the main cause of reduced degradation of dietary protein in the rumen and decreased N digestibility when red clover silage replaces grass silage in the diet (Dewhurst et al., 2003b; Merry et al.,

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2014; Halmemies et al., 2014). Huhtanen et al. (2014) suggested that the effect of PPO in red clover silage might decrease the post-rumen N digestibility compared with that of grass silage and concluded that the increased faecal N output when feeding red clover silage is not related to increased particle-associated crude protein (CP).

An environmental concern with forage-based milk production is that high forage diets are known to increase ruminal methane (CH₄) production compared with high concentrate diets (Johnson and Johnson, 1995). It has been suggested that legume silages could decrease CH₄ emissions from ruminants (Beauchemin et al., 2008; Lüscher et al., 2014). However, there is currently a lack of data on CH₄ emissions from ruminants fed legumes (Dewhurst, 2013).

Due to the possible increase in dietary NAN with more red clover silage in the diet of dairy cows and the positive production response to rapeseed in the diet, our starting hypothesis was that increasing the proportion of red clover silage in the diet would have a protein supplement sparing effect. Our second hypothesis was that increasing red clover silage in the diet decreases CH₄ emissions from dairy cows, by lowering the fibre content, and increases DMI. The overall aim of this study was to determine milk production responses and CH₄ emissions in dairy cows fed diets based on grass silage and low or high inclusion of red clover, mimicking the composition of primary and regrowth grass-red clover ley. Incremental levels of rapeseed expeller were also included in the diets, to investigate the optimal supplemental CP levels and possible concentrate sparing effects when feeding low or high red clover silage to dairy cows.

2. Materials and methods

The study was carried out with the permission of the Swedish Ethics Committee on Animal Research (Umeå, Sweden) and in accordance with Swedish laws and regulations and with the EU Directive 2010/63/EU on animal research.

2.1. Experimental design, animals and management

The study was conducted at the Röbbäcksdalen research station, which is part of the Swedish Infrastructure for Ecosystem Science within the Swedish University of Agricultural Sciences in Umeå (63°45' N; 20°17' E). A set of 32 lactating Swedish Red dairy cows (100 ± 34.4 days in milk; 32 ± 6.9 kg milk/day) were blocked according to parity and milk yield. Eight primiparous cows were blocked separately and older cows were blocked according to high, medium, or low yield. Within blocks, the cows were randomly assigned to treatments. The study was conducted as a cyclic change-over design (Davis and Hall, 1969), with eight treatments in a 2×4 factorial arrangement consisting of two grass-red clover silages and four levels of dietary CP fed to the cows in three experimental periods. Each period lasted 21 days and was divided into 14 d of adaptation and seven days of data recording and sampling. The cows were kept in an insulated, loose-housing barn and were milked twice a day, at 06:00 and 15:00. They were fed a total mixed ration *ad libitum*. A stationary feed mixer (Nolan A/S, Viborg, Denmark) processed the rations, which were then delivered with automatic feeder wagons into feed cribs three times per day, starting at 4:00, 10:00 and 17:00. During the trial, each cow had access to the same feed crib, shared with another experimental cow in pairs throughout the experiment.

2.2. Diets

The eight dietary treatments all had a forage-to-concentrate ratio of 60:40 on a DM basis (Table 1). The forage levels consisted of a mixture of either 70:30 (RC30) or 30:70 (RC70) grass silage and red clover silage on a DM basis. Furthermore, the red clover silage was a 50:50 (DM basis) mixture of a primary growth and regrowth ley from the same field.

Table 1
Proportions (g/kg DM) of different feed components in the experimental diets.

	Diets ^a							
	RC30				RC70			
	B	L	M	H	B	L	M	H
Grass silage	442	442	442	442	189	189	189	189
Clover silage, primary growth	95	95	95	95	221	221	221	221
Clover silage, regrowth	95	95	95	95	221	221	221	221
Crimped barley	316	242	168	95	316	242	168	95
Premix ^b	52	52	53	52	53	53	54	53
Rapeseed expeller ^c	0	74	147	221	0	74	147	221

^a Diets: RC30=30% red clover silage on DM basis in the forage ration and 70% grass silage, RC70=70% red clover silage in the forage ration and 30% grass silage, B = barley, no protein supplement, L = low level of rapeseed expeller, M = medium level of rapeseed expeller, H = high level of rapeseed expeller.

^b The premix (Fodercentralen, Umeå, Sweden) contained (g/kg feed) sugar beet pulp (250), sugar beet molasses (20), barley (166), oat (350), oat bran (50), NaCl (36), calcium carbonate (30), calcium-fat (40), mineral and vitamin concentrate (58).

^c Heat-moisture treated rapeseed expeller (Farmarin Öpex, Suomen Rehu, Hankkija Oy, Hyvinkää, Finland).

Four levels of concentrate CP were formulated. A basal concentrate (B; 130 g CP/kg DM) consisting of crimped barley and a premix (100 g/kg DM; Fodercentralen, Umeå, Sweden) was fed to fulfil mineral and vitamin requirements. Concentrate CP levels of 170, 210 and 250 g CP/kg DM (low (L), medium (M) and high (H), respectively) were achieved by replacing crimped barley with incremental levels of rapeseed expeller.

All silages were harvested in 2012 with a mower conditioner and precision-chop forage harvester (theoretical chopping length of 16–32 mm) and stored in separate bunker silos. The grass silage was a regrowth sward cut from a two-year-old ley of timothy and red clover (seed rate 80:20) fertilised with 40 kg N/ha (80 kg N/ha for the primary growth). The grass silage was harvested on 8 August and treated with an acid-based additive (Promyr TM XR 630, Perstorp, Sweden) at a rate of 3.5 L/tonne. The red clover silage came from a primary growth and a regrowth of a one-year-old pure red clover ley. It was fertilised with 30 kg N/ha for the primary growth, which was harvested on 6 July, while the regrowth was harvested on 15 August. Both primary growth and regrowth cuts were treated with the same acid-based additive as for the grass silage, at a rate of 6 L/tonne. The crimped barley (59.8% DM) was rolled using a mill (Murska 1400 S2×2, Murska, Ylivieska, Finland) adjusted to 0.3 mm between the rollers, treated with 3.5 L/tonne of propionic acid and stored in airtight bags (1.6 m×60 m, Ltd Rani Plast Oy, Terjärv, Finland). The treated rapeseed expeller was a commercial feed (Öpex, Mildola Ltd, Espoo, Finland), made by pressing double-zero rapeseeds (low in glucosinolates and erucic acid) under heating to extract the oil and then applying a heat-moisture treatment to the expeller.

2.3. Animal recordings

Feed intake was recorded daily in Roughage Intake Control feeders (Insentec, B.V., Marknesse, the Netherlands), and data from day 15–21 in each period were used in the statistical analysis. Body weight was measured after morning milking on day 19–21 in each period. Milk yield was recorded daily with gravimetric milk recorders (SAC, S.A. Christensen and Co Ltd, Kolding, Denmark), and data from day 15–21 were used in the statistical calculations. Milk samples were collected at four consecutive milking, from the afternoon milking of day 19 until the morning milking of day 21 in each period. The morning samples were pooled together, as were the evening samples, and then stored at 8 °C until sent for analysis.

The mass flux of CH₄ and carbon dioxide (CO₂) in exhaled air from individual animals was recorded by two portable open-circuit head

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