Effect of silage botanical composition on ruminal biohydrogenation and transfer of fatty acids to milk in dairy cows

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ABSTRACT

Ruminal biohydrogenation and transfer of fatty acids (FA) to milk were determined for 4 silages with different botanical compositions using 4 multiparous Norwegian Red dairy cows [(mean $\pm$ SD) 118 $\pm$ 40.9 d in milk, 22.5 $\pm$ 2.72 kg of milk/d, 631 $\pm$ 3.3 kg of body weight, 3.3 $\pm$ 0.40 points on body condition score at the start of the experiment] fitted with rumen cannulas. Treatments consisted of 4 experimental silages: a mix of the first and third cut of organically managed short-term grassland with timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.; 2 yr old; ORG-SG); organically managed long-term grassland with a high proportion of unsown species (6 yr old; ORG-LG); conventionally managed ley with perennial ryegrass (*Lolium perenne* L.; CON-PR); and conventionally managed ley with timothy (CON-TI). The herbage was cut, wilted, and preserved with additive in round bales and fed at 0.90 of ad libitum intake. A barley (*Hordeum vulgare* L.) concentrate constituted 300 g/kg of dry matter of the total feed offered. A Latin square design (4 $\times$ 4) with 3-wk periods and the last week in each period used for sampling was implemented. Omasal flows of FA were measured using Yb acetate, Cr-EDTA, and the indigestible neutral detergent fiber fraction as indigestible markers. The composition of FA was analyzed in feed, omasal digesta, and milk. Compared with ORG-LG, ORG-SG had a higher herbage proportion of red clover (0.36 vs. 0.01) and lower proportions of timothy (0.42 vs. 0.18), smooth meadowgrass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* Huds.), white clover (*Trifolium repens* L.), dandelion (*Taraxacum* spp.), and creeping buttercup (*Ranunculus repens* L.). The silages were well preserved. The concentration of neutral detergent fiber was higher and the concentration of Kjeldahl-N was lower for CON-TI than for the other

silages. Silage type had no effect on dry matter intake, but milk yield was lower for CON-TI than for the other silages. Apparent biohydrogenation of C18:3n-3 was lower for ORG-SG (932 g/kg) than for ORG-LG (956 g/kg), CON-PR (959 g/kg), and CON-TI (958 g/kg). Compared with the grass-based silages, ORG-SG and ORG-LG resulted in higher omasal flows of C18:1 *trans* FA and higher milk fat proportions of C18:1 *trans* FA and C18:2 *cis*-9,*trans*-11. Apparent recovery of C18:3n-3 in milk was higher for ORG-SG (61 g/kg) than for ORG-LG (33 g/kg), CON-PR (34 g/kg), and CON-TI (38 g/kg), and milk fat proportion of C18:3n-3 was higher for ORG-SG than for CON-TI. Milk fat proportions of C16:0 were lower for ORG-SG and ORG-LG compared with those for CON-PR and CON-TI. It was concluded that high proportions of red clover and other dicotyledons in the silages affected ruminal biohydrogenation and increased milk fat proportions of beneficial FA.

Key words: botanical composition, silage, biohydrogenation, fatty acid

INTRODUCTION

The healthfulness of milk and dairy products has been challenged because of their relatively high concentrations of SFA, which is considered to be associated with cardiovascular diseases (Kato et al., 1973). However, recent research has revealed several ingredients in milk that may be beneficial for human health. Among these bioactive components are FA such as C18:2 *cis*-9,*trans*-11, C18:2n-6, C18:3n-3, C20:5n-3, and C22:6n-3 (Simopoulos, 1991; Larsson et al., 2005). Nutritionists, who also focus on the dietary balance between n-6 and n-3 PUFA, indicate that the n-6:n-3 ratio is too high in Western diets (Simopoulos, 2002).

Forage plants contain high proportions of unsaturated FA; for example, the proportion of C18:3n-3 in fresh grass constitutes approximately 0.50 of the total FA concentration. The FA composition of forages is affected by plant species (Dewhurst et al., 2001), stage of

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maturity (Vanhatalo et al., 2007), preservation method, and environmental factors (Boufaïed et al., 2003). Unsaturated feed FA are extensively biohydrogenated in the rumen, resulting in a range of biohydrogenation intermediates and C18:0, which together with de novo-synthesized FA result in a high proportion of SFA in ruminant products. Thus, a reduction in biohydrogenation rate will increase the proportion of unsaturated FA in ruminant products.

The extent of ruminal biohydrogenation of C18:2n-6 and C18:3n-3 may be reduced by increasing the rumen passage rate, as observed for white clover (*Trifolium repens* L.) relative to grass (Dewhurst et al., 2003a); by the activity of secondary plant metabolites, as observed in vitro for silages containing red clover (*Trifolium pratense* L.; Lee et al., 2004); or by the type and amount of feed PUFA with differential toxicities to rumen microbes (Maia et al., 2007). Changes in rumen microbe composition may lead to incomplete biohydrogenation and the accumulation of intermediates, such as C18:1 *trans*-11 (Lourenço et al., 2010), which finally appear in the milk (Harfoot and Hazlewood, 1997). Rumen microbes not only biohydrogenate unsaturated feed FA, but also produce SFA (C2:0 through C16:0) and odd-chain FA de novo in the rumen (Kaneda, 1991). Short-chain FA are used as an energy source and for de novo synthesis of FA in the mammary gland, where mainly C2:0 is elongated with C2 units and some of the terminated FA are Δ^9 -desaturated, incorporated into triacylglycerides, and organized into milk fat globules (Mansbridge and Blake, 1997; Vlaeminck et al., 2006).

Higher proportions of C18:3n-3 and a higher n-3:n-6 FA ratio have been found in milk produced on red clover and white clover silages than on grass (Poaceae) silages (Dewhurst et al., 2003b; Höjer et al., 2012). Polyphenol oxidase (EC 1.14.18.1) activity in red clover may protect forage lipids in phenol-bound proteins from ruminal biohydrogenation (Lourenço et al., 2008). A similar proportion of C18:3n-3 and a higher proportion of C18:2 *cis*-9,*trans*-11 in milk fat have been observed when feeding silages prepared from species-rich seminatural grassland when compared with intensively managed grassland, despite a reduced dietary intake of C18:3n-3 (Lourenço et al., 2005). The higher milk fat proportion of C18:2 *cis*-9,*trans*-11 was related to a partial inhibition of rumen biohydrogenation when feeding species-rich silage. Thus, silages with high proportions of red clover, nonlegume (Fabaceae) dicotyledons, or grasses have different effects on rumen biohydrogenation and milk FA composition. The objective of this experiment was to determine the effect of silage botanical composition on ruminal biohydrogenation and the transfer of feed FA to milk.

MATERIALS AND METHODS

Animals, Feeds, Experimental Design, Herbage Botanical Composition and Stage of Development, and Feed Sampling

Four lactating multiparous Norwegian Red dairy cows [(mean $\pm$ SD) 118 $\pm$ 40.9 DIM, 22.5 $\pm$ 2.72 kg of milk/d, 631 $\pm$ 3.3 kg of BW, 3.3 $\pm$ 0.40 points on BCS], fitted with 10-cm rumen cannulas (Bar Diamond Inc., Parma, ID), were allocated at random to 4 experimental diets in a 4 $\times$ 4 Latin square. Each period lasted for 21 d, of which the first 14 d were used for adaptation and the last 7 d were used for sampling. On d 1 through 11, the cows were fed the experimental silages ad libitum plus a barley (*Hordeum vulgare* L.)-based concentrate (g/kg): barley, 933; molasses, 50; and mineral premix, 17. The mineral premix in the concentrate contained (g/kg) Ca, 110; P, 65; Mg, 90; Na, 95; Cl, 143; S, 9; Fe, 0.494; Mn, 3.00; Zn, 4.00; Cu, 0.900; Co, 0.021; I, 0.152; mineral-Se, 0.030; and yeast-Se, 0.030 (Vilomix; Normin, Hønefoss, Norway). The concentrate allotment was adjusted daily to constitute 0.30 of total DMI, based on recordings of silage DMI from the previous day. On d 12 through 21, silage and concentrate were limited to 0.90 of the average intake for d 1 through 11, with the aim of reducing daily feed residues and keeping the forage:concentrate ratio as stable as possible. Daily mixed feed rations were fed as equal meals at 0600, 1200, 1800, and 0000 h, and feed residues were recorded daily at 0930 h.

The first cut (June 5), second cut (July 14), and third cut (September 8, 2009) of organically managed (Council of European Union, 2007) short-term grassland (**ORG-SG**), organically managed long-term grassland (**ORG-LG**), conventionally managed perennial ryegrass (*Lolium perenne* L.) ley (**CON-PR**), and conventionally managed timothy (*Phleum pratense* L.) ley (**CON-TI**) were harvested with a disc mower in Ås, Norway (N 59.7°, E 10.8°). After wilting to a DM concentration of approximately 300 g of DM/kg, the herbage was ensiled in round bales with an additive at a rate of 4 to 5 L/t of fresh weight (GrasAAT N Lacto; ADDCON Nordic AS, Porsgrunn, Norway). First-cut and third-cut silages were chopped and mixed in a 1:1 ratio on a DM basis before feeding. Yields from the second cut were low and were therefore not included in the silage mixtures.

The ORG-SG was established in 2007 (timothy var. 'Grindstad' and red clover var. 'Bjursele') and fertilized with 25 t/ha of farmyard manure in spring 2009. The ORG-LG was established in 2003 [perennial ryegrass, white clover, smooth meadowgrass (*Poa pratensis* L.)] and fertilized with 20 t/ha of farmyard manure

in spring 2009. The CON-PR (var. 'Napoleon') and CON-TI (var. 'Grindstad') were established in 2007 and fertilized with mineral fertilizer supplying 88 kg of N/ha in spring and 84 kg of N/ha after the first and second cuts, respectively.

Botanical composition of the herbage was estimated by hand-clipping six 0.5-m² squares per field before each cut, separated by hand into individual species. The prevailing stage of development was estimated for timothy and perennial ryegrass by using the scheme of Gustavsson (2011). Herbage yields were measured by hand-clipping six 0.5-m² squares per field before each cut and oven drying (103°C for 24 h).

Silages, concentrates, and feed residues within treatment were sampled daily (1 kg) from d 15 through 21 in each period and stored frozen (−20°C). Silage, concentrate, and feed residue samples were pooled and mixed for each type and period. Silage samples intended for analysis of DM, VFA, lactic acid, NH₃-N, ethanol, and pH were stored frozen (−20°C).

Silage, concentrate, and feed residue samples intended for analysis of ash, Kjeldahl-N, crude fat, NDF, FA composition, and starch and determination of the indigestible NDF (iNDF) fraction (except feed residues), were freeze-dried (Christ LCM-2, β 1–16 and Christ LOC-1m, α 1–4, Martin Christ, Osterode am Harz, Germany; Hetosicc, Birkerød, Denmark) and ground on a cutting mill with a grind size of 1.0 mm, except for starch (0.5 mm) and iNDF (1.5 mm; Retsch SM 100; Retsch GmbH, Haan, Germany) before analysis. The experiment was carried out in agreement with the laws and regulations controlling experiments on live animals in Norway under the supervision of the Norwegian Animal Research Authority.

Administration of Flow Markers, Flow Measurements, and Ruminal Sampling

The flow of digesta through the omasal canal was estimated using the iNDF fraction as an internal marker for large particles, Yb acetate as a particulate marker for small particles, and Cr-EDTA as a liquid marker (Faichney, 1975). Ytterbium acetate was mixed with distilled water and stirred overnight, and then mixed with Cr-EDTA (Binnerts et al., 1968; 0.877 g of Yb/L, 0.894 g of Cr/L) before infusion into the rumen at a rate of 2.3 g of Yb/d and 2.4 g of Cr/d. Ruminal infusions began on d 8, with priming doses of 1.3 g of Yb and 1.3 g of Cr dissolved in 1.5 L water, and continued at a regular rate until d 20.

Digesta (500 mL) entering the omasal canal were sampled according to the method of Huhtanen et al. (1997) as modified by Ahvenjärvi et al. (2001). In total,

12 digesta samples were collected per animal and period on d 17 (at 0500, 1100, 1700, and 2300 h), d 18 (at 0300, 0900, 1500, and 2100 h), and d 19 (at 0100, 0700, 1300, and 1900 h) to cover each second hour during a 24-h period. The samples were stored frozen (−20°C) until thawed in cold water for 18 h and then mixed, and subsamples of approximately 4.7 kg were added to a solution of 200 g of NaCl (0.9%) before separation of the large particle phase (nylon filter 100-μm pore size), small particle phase (centrifuged at 10,000 × *g* for 15 min at 4°C), and liquid phase. Freeze-dried samples of the large particle phases and the small particle phases were ground (<1.0-mm grind size) before analysis of DM, ash, NDF, Kjeldahl-N, FA composition, Yb, and Cr and for determination of the iNDF fraction (<1.5 mm), as described for the experimental feeds. The freeze-dried samples of the liquid phases were stored in plastic bags (−20°C) until analysis of DM, ash, Kjeldahl-N, FA composition, NH₃-N, Yb, Cr, and VFA.

Rumen fluid (10 mL) was sampled through the rumen cannula every second hour beginning at 0600 h until 1800 h on d 21 in each period and preserved with 0.5 mL of 98% formic acid. The samples were stored at 4°C until analysis of NH₃-N, and VFA. On d 21, pH was measured in the rumen over a 24-h period and logged every 10 min with a WTW pH340 pH probe (WTW, Weilheim, Germany).

Animal Measurements

The cows were weighed on 2 consecutive days at the beginning and end of each experimental period. Body condition score was estimated visually by the method of Edmonson et al. (1989) by using a 5-point scale (1 = emaciated to 5 = severely overconditioned) with 0.25-unit increments at the beginning of the experiment and at the end of each period.

Feces and Urine Collection

Total fecal and urine collections were done on d 18 through 20. Feces were collected in buckets quantitatively 3 times per day (1400, 2200, and 0600 h). Buckets with feces were stored chilled during each collection day. After the last collection each day, the feces were mixed, sampled, and stored frozen (−20°C). After each period, the feces were thawed and split; one sample was analyzed for DM and 1 sample was freeze-dried and ground (<1-mm grind size) before analysis of NDF, Kjeldahl-N, starch, Yb, Cr, and iNDF (<1.5 mm). For urine collection, the cows were fitted with funnels that led to bottles filled with 0.5 L (10%) of sulfuric acid. Urine was collected 3 times per day (1400, 2200, and

0600 h) and 10% of each daily urine collection was frozen (-20°C). After each period, urine samples were thawed, mixed, and stored frozen until analysis of Cr.

Milk Recording and Sampling

Cows were milked twice daily (0630 and 1530 h), and on d 18 through 20, aliquots were sampled and stored at 0 to 2°C until samples were pooled and mixed for each cow at the end of each period. Samples intended for analysis of gross composition and concentrations of urea and FFA were preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol; D&F Inc., Dublin, CA). Samples intended for analysis of FA composition were stored frozen (-20°C) until analysis.

Chemical Analyses

Freeze-dried samples of silages, concentrates, omasal digesta (large and small particle phases), and feces were analyzed for DM (103°C for 24 h) and ash (550°C for 24 h); Kjeldahl-N (Kjeltec 2400; Foss, Hillerød, Denmark), using a Cu catalyst; crude fat after hydrolysis with petroleum ether on an Accelerated Solvent Extractor (ASE200; Dionex, Sunnyvale, CA); NDF on an Ankom220 fiber analyzer (Ankom Technology, Macedon, NY; Mertens, 2002), using sodium sulfite and α amylase; and starch (McCleary et al., 1994), with the modification of using heat-stable α -amylase. The iNDF fraction in these samples was determined in 2 cows after in vivo incubation for 288 h in nylon bags (pore size $17\text{ }\mu\text{m}$). After incubation, the bags were rinsed in a washing machine, extracted in NDF solution at 100°C for 1 h, and dried at 60°C .

The liquid phase of omasal digesta was analyzed for DM, Kjeldahl-N, and $\text{NH}_3\text{-N}$ (FIAstar 5010; Foss, Hillerød, Denmark) and the liquid phase of omasal digesta and rumen fluid were analyzed for VFA by gas chromatography (Restek Stabilwax-DA 30-m column; Restek Corporation, Bellefonte, PA).

Samples of silages not dried were analyzed for VFA, lactic acid, $\text{NH}_3\text{-N}$, and ethanol by HPLC using a VA 300/7.8 Nucleogel Ion 300 OA column (Machery-Nagel, Düren, Germany) at 50°C (mobile phase, 0.010 mmol/L of sulfuric acid at 0.6 mL/min) with a UV spectrophotometric detector for lactic acid and a refractive index detector for other acids and ethanol. Ammonia-N was analyzed with MAN-TECH PC-titrate (Guelph, ON, Canada) using an Orion ion analyzer 901, and pH was analyzed with a Thermo Orion 420A + pH meter with an Orion 9107BN electrode (Thermo Scientific, Beverly, MA) by Eurofins (Moss, Norway).

Dried samples of omasal digesta phases, infusion liquids, and urine were analyzed for Cr according to

the method described by Udén et al. (1980), with the following modifications: samples (100 mg) were combusted in scintillation vials, dissolved in 0.75 mL of phosphoric acid and 1 mL of potassium bromate, and boiled for 10 min. Samples (200 mg) of omasal digesta phases and infusion liquids for Yb analysis were dissolved in scintillation vials and boiled for 30 min.

Lipids in feed and omasal digesta were analyzed following an acid Bligh and Dyer extraction (Jensen, 2008). Samples were acidified in 80°C (3 mol/L) HCl for 1 h and extracted in a mixture of chloroform and methanol (Bligh and Dyer, 1959). Lipids in milk were extracted according to the method of Bligh and Dyer (1959). Lipid extracts were quantified as FA methyl esters, as described by Jensen and Nielsen (1996), using a gas chromatograph (Hewlett Packard 6890 series; Agilent Technologies, Palo Alto, CA) equipped with an automatic on-column injector (Hewlett Packard 7673; split ratio of 4.325:1), a capillary column of $30\text{ m} \times 320\text{ }\mu\text{m}$ i.d., $0.25\text{-}\mu\text{m}$ film thickness (Omegawax; Supelco 4-293-415; Sigma-Aldrich, St. Louis, MO), and a flame-ionization detector. The internal standard used was C17:0. Milk gross composition and concentrations of urea and FFA were analyzed by Fourier transform infrared technology (MilkoScan 6000 FTIR; Foss, Hillerød, Denmark).

Calculations and Statistics

Dry matter analyses in silage and the liquid digesta phase were corrected for volatile compounds according to the method of Åkerlind et al. (2011). Digesta flows were calculated using the 3-marker system according to the reconstitution techniques of Faichney (1975) and Armentano and Russell (1985).

The recoveries of markers in feces were generally low for iNDF (mean 0.60) and Cr (0.73) and high for Yb (1.13). Further, marker recoveries were low for CON-PR compared with the mean of the other diets in all periods (CON-PR as a proportion of the mean of the other diets: iNDF, 0.71; Yb, 0.79; Cr, 0.78). This was associated with low collection of fecal DM compared with the other diets in all periods (CON-PR as a proportion of the mean of the other diets: 0.71). Therefore, the amount of feces for CON-PR was adjusted to give the same recovery for Cr as for the mean of ORG-SG, ORG-LG, and CON-TI in each period by dividing fecal amounts by 0.671 in period 1, 0.912 in period 2, 0.727 in period 3, and 0.804 in period 4. This lowered the estimates of total tract digestibility by a factor of 0.93 for CON-PR.

The apparent ruminal biohydrogenation of dietary FA was calculated in 2 ways: (1) $1,000 - 1,000 \times (\text{omasal flow of FA}/\text{intake of FA})$, and (2) according to the

method of Wu et al. (1991): $1,000 - 1,000 \times [(omasal \text{ flow of FA} / omasal \text{ flow of total C18-FA}) / (\text{intake of FA} / \text{intake of total C18-FA})]$. It was assumed that no mobilization or transport of FA to adipose tissue happened as the cows were in midlactation and that the plane of nutrition was balancing milk yield.

Feed intake, digestibility, milk yield, milk composition, and ruminal biohydrogenation variables were analyzed statistically using the mixed model procedure by SAS (SAS Institute, 2009). The following statistical model was used:

$$Y_{ijk} = \mu + S_i + P_j + c_k + e_{ijk},$$

where Y were the individual dependent variables ($k = 1$ through 16) and μ was the average of all observations, S was the fixed effect of silage type ($i = 1$ through 4; where 1 = silage of ORG-SG, 2 = ORG-LG, 3 = CON-PR, 4 = CON-TI), P was the fixed effect of period ($j = 1$ through 4), c was the random effect of cow ($k = 1$ through 4), and e_{ijk} were the random residual errors, assumed to be independent, and $N(0, \sigma_e^2)$. Contrasts were calculated for the effects of ORG-SG vs. ORG-LG, CON-PR vs. CON-TI, and ORG-SG + ORG-LG vs. CON-PR + CON-TI. Differences between least squares means were estimated with the Tukey-Kramer test.

RESULTS

Botanical Composition and Chemical Content of the Experimental Feeds

The ORG-SG was characterized by high proportions of timothy and red clover and low proportions of nonlegume dicotyledons (Table 1). Compared with ORG-SG, ORG-LG had higher proportions of smooth meadowgrass, meadow fescue (*Festuca pratensis* L.), white clover, dandelion (*Taraxacum* spp.), and creeping buttercup (*Ranunculus repens* L.). At the first cutting, timothy and perennial ryegrass plants were in development stage 49 (the tip of the inflorescence just below the flag leaf blade base). At the third cutting, timothy plants were in development stages 33 through 35 (stem elongation, node 3 to 5 palpable) and perennial ryegrass was in development stage 1 (leaf development). Total forage yields were lowest for ORG-LG and highest for CON-PR.

The experimental silages were well preserved, but higher concentrations of lactic acid and acetic acid in silages from ORG-LG and CON-PR indicated more extensive fermentation than in silages from ORG-SG and CON-TI, probably caused by lower DM concentra-

tions (Table 2). Kjeldahl-N concentration was lowest and NDF concentration was highest in silage CON-TI. Compared with ORG-SG and CON-TI, silages from ORG-LG and CON-PR had higher concentrations of total FA, mainly because of the higher concentrations of C18:3n-3. Compared with the silages, the barley concentrate had a lower fat concentration but higher concentrations of C16:0, C18:1 *cis*-9, and C18:2n-6.

Feed Intake, Rumen Metabolism, Feed Nutrient Digestibility Values, and Milk Production

Silage DMI did not differ between diets (Table 3). Only small differences were found in concentrations of VFA in rumen fluid and in pH. Milk yield was lower ($P < 0.001$) for CON-TI than for the other silages. Rumen and total tract digestibility values of nutrients were not affected by silage type (Table 4).

Apparent Ruminal Biohydrogenation of FA

The intake of C18:2n-6 was highest ($P = 0.005$) when ORG-LG was fed, and that of C18:3n-3 was highest ($P = 0.008$) when ORG-LG or CON-PR was fed (Table 5). The omasal flows of C18:0 ($P = 0.009$) and C18:1 *trans* FA ($P < 0.001$) were lower for ORG-SG than for ORG-LG. The omasal flow of C18:1 *trans* FA was almost doubled ($P < 0.001$) for ORG-SG compared with ORG-LG. The omasal flows of C18:0 ($P < 0.001$) and C18:1 *trans* FA ($P < 0.001$) in total C18 FA were lower for ORG than for CON silages. Apparent biohydrogenation of C18:3n-3 was lower ($P = 0.004$) for ORG-LG than for the other silages, and the apparent biohydrogenation of total unsaturated C18 FA was lower ($P < 0.001$) for ORG than for CON silages. The calculation of apparent biohydrogenation in total C18-FA resulted in similar effects of diets, but slightly lower values.

Milk FA Composition and Transfer of FA to Milk

Milk fat produced on ORG silages had higher proportions of C18:1 *trans* FA ($P < 0.001$), C18:2 *cis*-9, *trans*-11 ($P < 0.001$), and C18:2 *trans*-10, *cis*-12 ($P = 0.005$) and lower proportions of C16:0 ($P = 0.004$) than milk fat produced on CON silages (Table 6). The milk fat proportion of C18:3n-3 was highest ($P = 0.01$) for ORG-SG, and apparent recovery of C18:3n-3 in milk from feed was also highest ($P < 0.001$) for ORG-SG. Apparent recovery of C18 FA in the omasum from feed was low (720 to 817 g/kg) but was not affected by silage type. The proportions of the apparently de novo-synthesized sum of FA in milk fat were lower ($P = 0.002$) for ORG than for CON.

Table 1. Total herbage yield (n = 12), DM concentration after wilting (n = 8), and proportion of botanical families and species (n = 12) in the experimental silages as fed in a 1:1 mixture of the first and third cut of silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass (CON-PR), or conventionally managed timothy (CON-TI)

Item	Treatment			
	ORG-SG	ORG-LG	CON-PR	CON-TI
Total herbage yield, tonne of DM/ha	5.70	3.28	10.17	6.00
DM after wilting, g of DM/kg	328	278	301	340
Botanical composition, g of DM/kg of DM				
Grass family (Poaceae)	521	626	875	960
Timothy (<i>Phleum pratense</i> L.)	420	176	0	957
Perennial ryegrass (<i>Lolium perenne</i> L.)	12	12	873	0
Smooth meadowgrass (<i>Poa pratensis</i> L.)	3	257	0	0
Meadow fescue (<i>Festuca pratensis</i> Huds.)	0	107	0	0
Common couch [<i>Elytrigia repens</i> (L.) Desv. ex Nevski]	85	38	2	3
Rough meadowgrass (<i>Poa trivialis</i> L.)	1	27	0	0
Other grasses ¹	0	8	0	0
Legume family (Fabaceae)	391	104	62	2
Red clover (<i>Trifolium pratense</i> L.)	363	14	2	0
White clover (<i>Trifolium repens</i> L.)	26	90	60	1
Other legumes ²	1	0	0	1
Other botanical families ³	30	233	0	0
Dandelion (<i>Taraxacum</i> spp.)	4	95	0	0
Creeping buttercup (<i>Ranunculus repens</i> L.)	6	90	0	0
Northern dock (<i>Rumex longifolius</i> DC.)	12	19	0	0
Greater plantain (<i>Plantago major</i> L.)	2	16	0	0
Other species ⁴	6	13	0	0
Dead material	59	37	62	38
Number of spp.	20	23	12	5

¹*Deschampsia cespitosa* (L.) P. Beauv., *Poa annua* L., *Festuca rubra* L., *Agrostis capillaris* L.

²*Vicia cracca* L., *Trifolium hybridum* L.

³Ranunculaceae, Asteraceae, Polygonaceae, Plantaginaceae, Caryophyllaceae, Scrophulariaceae, Brassicaceae.

⁴*Ranunculus acris* L., *Achillea millefolium* L., *Cirsium helenioides* (L.) Hill, *Stellaria graminea* L., *Cerastium fontanum* Baumg., *Stellaria media* (L.) Vill., *Veronica serpyllifolia* L., *Thlaspi arvense* L., *Capsella bursa-pastoris* (L.) Medik.

DISCUSSION

Methodical Considerations in Flow Measurements

Issues with marker recovery in the feces were observed in the present study. Average recovery of iNDF (634 g/kg) and Cr (771 g/kg) were low, and the recovery of Yb was higher than the intake (1,203 g/kg). This implies that omasal flow measurements should have been corrected for the fecal recovery of markers. However, incomplete recovery of markers in feces may have been caused by different sources: erroneous analysis by using atomic absorption spectrometry because of interfering compounds in digesta and feces (Siddons et al., 1985; Holt, 1993), inconsistent infusion rates, occasional leakage from cannulas (Ahvenjärvi et al., 2003), or inaccurate amounts of feces collected. The latter potential source of error can probably be excluded because the total tract digestion coefficient of starch was, as expected, almost 1 for a concentrate based on barley (Waldo, 1973). Moreover, the NDF total tract digestibility of 715 to 751 g/kg was high, but expected, because the forage plants were immature (low NDF concentration) at harvesting, in accordance with others (Randby et al., 2012). Accordingly, the relatively low proportion di-

gested in the rumen of NDF digested totally (810 g/kg) compared with other studies (Huhtanen et al., 2010) was most likely caused by other sources of error. In 3-marker systems, inaccuracy of Co or Yb administration has minor effects on calculated NDF flow, whereas overestimation of iNDF administration results in overestimation of calculated NDF flows and hence underestimates NDF digestibility in the rumen (Ahvenjärvi et al., 2003), consistent with our results. Unrepresentative sampling of omasal digesta (Ahvenjärvi et al., 2001) is another plausible explanation for the low rumen digestibility obtained for NDF.

Corrections for fecal recovery of markers assume an equal correction for fecal and omasal samples, which may not be the case. Thus, assessed on an overall evaluation of omasal and fecal digestibility values, the authors concluded that the most reliable results were obtained by using uncorrected flow measurements.

Silage Chemical Composition and Animal Performance

The low milk yield for CON-TI may be explained by the lower silage DMI, lower concentration of protein,

Table 2. Dry matter, chemical composition, fermentation products, and FA concentration in the experimental silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass ley (CON-PR), and conventionally managed timothy ley (CON-TI) and concentrate (mean $\pm$ SD; n = 4)

Item	Treatment				Concentrate
	ORG-SG	ORG-LG	CON-PR	CON-TI	
DM, g/kg	325 $\pm$ 4.1	272 $\pm$ 4.4	293 $\pm$ 6.3	320 $\pm$ 19.6	901 $\pm$ 2.1
Chemical composition, g/kg of DM					
Ash	86 $\pm$ 2.7	105 $\pm$ 28.2	94 $\pm$ 2.6	75 $\pm$ 2.4	45 $\pm$ 4.0
NDF	428 $\pm$ 8.4	434 $\pm$ 9.4	422 $\pm$ 11.6	534 $\pm$ 11.6	168 $\pm$ 3.5
Indigestible NDF	100 $\pm$ 3.0	88 $\pm$ 15.6	79 $\pm$ 1.3	95 $\pm$ 3.8	45 $\pm$ 1.1
Kjeldahl-N	23 $\pm$ 1.4	22 $\pm$ 0.3	24 $\pm$ 1.0	18 $\pm$ 0.4	20 $\pm$ 0.1
Crude fat	32 $\pm$ 5.8	42 $\pm$ 2.9	42 $\pm$ 3.8	39 $\pm$ 2.2	20 $\pm$ 2.1
Starch	34 $\pm$ 3.6	18 $\pm$ 1.7	22 $\pm$ 2.9	32 $\pm$ 26.2	510 $\pm$ 11.3
VFA and lactic acid, g/kg of DM					
Lactic acid	31.0 $\pm$ 2.04	62.9 $\pm$ 1.25	60.8 $\pm$ 2.06	34.4 $\pm$ 10.74	—
Acetic acid	6.3 $\pm$ 0.56	10.0 $\pm$ 0.39	9.9 $\pm$ 0.49	6.4 $\pm$ 0.47	—
Butyric acid	0.8 $\pm$ 0.28	0.9 $\pm$ 0.21	0.6 $\pm$ 0.08	0.5 $\pm$ 0.34	—
Formic acid	6.7 $\pm$ 0.30	7.3 $\pm$ 1.56	7.5 $\pm$ 1.84	4.1 $\pm$ 1.08	—
Propionic acid	0.0	0.0	0.0	0.0	—
NH ₃ -N, g/kg of DM	1.3 $\pm$ 0.10	1.6 $\pm$ 0.06	2.0 $\pm$ 0.21	1.6 $\pm$ 0.11	—
NH ₃ -N, g/kg of N	60.8 $\pm$ 2.99	81.5 $\pm$ 7.14	83.3 $\pm$ 7.54	96.3 $\pm$ 11.24	—
Ethanol, g/kg of DM	14.8 $\pm$ 2.13	18.6 $\pm$ 2.69	19.7 $\pm$ 2.65	16.1 $\pm$ 2.12	—
pH	4.5 $\pm$ 0.00	4.4 $\pm$ 0.06	4.5 $\pm$ 0.05	4.7 $\pm$ 0.17	—
FA, g/kg of DM					
C16:0	2.27 $\pm$ 0.185	2.90 $\pm$ 0.154	2.76 $\pm$ 0.093	1.93 $\pm$ 0.163	5.26 $\pm$ 0.140
C18:0	0.29 $\pm$ 0.018	0.30 $\pm$ 0.017	0.33 $\pm$ 0.010	0.24 $\pm$ 0.010	0.45 $\pm$ 0.019
C18:1 <i>cis</i> -9	0.52 $\pm$ 0.050	0.84 $\pm$ 0.043	0.47 $\pm$ 0.006	0.48 $\pm$ 0.024	3.23 $\pm$ 0.228
C18:2n-6	2.56 $\pm$ 0.208	3.81 $\pm$ 0.457	2.37 $\pm$ 0.101	2.40 $\pm$ 0.188	11.79 $\pm$ 0.389
C18:3n-3	6.78 $\pm$ 0.541	9.43 $\pm$ 0.802	9.90 $\pm$ 0.686	6.80 $\pm$ 0.764	1.10 $\pm$ 0.044
Total FA	13.66 $\pm$ 1.073	18.85 $\pm$ 1.584	17.53 $\pm$ 0.745	13.15 $\pm$ 1.059	22.52 $\pm$ 0.867

Table 3. Dry matter intake, rumen fermentation products and pH, milk yield, and milk gross composition for cows fed the experimental silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass ley (CON-PR), or conventionally managed timothy ley (CON-TI; n = 4)

Item	Treatment				SED ¹	Silage	P-value		
	ORG-SG	ORG-LG	CON-PR	CON-TI			ORG-SG vs. ORG-LG ²	CON-PR vs. CON-TI ²	ORG vs. CON ²
DMI, kg of DM/d									
Silage	12.5	12.5	12.2	11.6	0.52	NS	NS	NS	NS
Concentrate	5.4	5.4	5.2	5.1	0.18	NS	NS	NS	0.08
Rumen VFA, mmol/L									
Acetic acid	87.0	73.2	73.4	77.2	5.7	NS	0.05	NS	NS
Propionic acid	18.6	16.8	17.9	16.8	1.8	NS	NS	NS	NS
Isobutyric acid	0.8	0.8	0.9	0.7	0.1	NS	NS	0.09	NS
Butyric acid	12.9	17.4	12.8	13.0	3.2	NS	NS	NS	NS
Isovaleric acid	2.0	1.9	2.3	1.7	0.2	NS	NS	0.05	NS
Valeric acid	2.2	2.0	2.2	1.9	0.2	NS	NS	NS	NS
Sum of acids	123.6	112.0	109.4	111.3	8.6	NS	NS	NS	NS
Rumen pH ³	6.27	6.37	6.22	6.37	0.159	NS	NS	NS	NS
Rumen NH ₃ -N, mmol/L	45.6 ^c	68.4 ^{bc}	92.1 ^{ab}	54.9 ^c	8.5	0.01	0.04	0.005	0.03
Milk, kg/d	17.3 ^a	18.0 ^a	17.5 ^a	15.9 ^b	0.25	<0.001	0.05	<0.001	0.002
ECM, kg/d	18.9	19.2	19.0	17.8	0.56	NS	NS	NS	NS
Milk gross composition									
Fat, g/kg	45.6	44.3	45.7	48.6	1.90	NS	NS	NS	NS
Protein, g/kg	36.8	36.3	36.4	36.3	0.60	NS	NS	NS	NS
Lactose, g/kg	45.7	46.0	46.1	45.2	0.27	0.08	NS	0.02	NS
Urea, mmol/L	4.00 ^c	4.15 ^{bc}	5.18 ^{ab}	3.60 ^c	0.317	0.01	NS	0.003	NS
FFA, mEq/L	0.85	0.65	1.00	0.90	0.346	NS	NS	NS	NS

^{a-c}Means within a row with different superscripts differ (Tukey-Kramer test, $P < 0.05$).¹Standard error of the difference.²Contrasts.³Means of 24 h; 4 means excluded because of problems with the pH meter.

Table 4. Rumen digestibility (g/kg) of NDF and total tract digestibility of NDF and starch for cows fed the experimental silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass ley (CON-PR), or conventionally managed timothy ley (CON-TI; n = 4)

Item	Treatment				SED ¹	Silage	P-value		
	ORG-SG	ORG-LG	CON-PR	CON-TI			ORG-SG vs. ORG-LG ²	CON-PR vs. CON-TI ²	ORG vs. CON ²
Rumen digestibility									
NDF	559	585	624	602	25.9	NS	NS	NS	0.07
Total tract digestibility									
NDF	725	715	751	735	25.3	NS	NS	NS	NS
Starch	995	996	996	996	1.3	NS	NS	NS	NS

¹Standard error of the difference.

²Contrasts.

and higher concentration of NDF compared with the other silages. The high concentration of iNDF in ORG-SG silage could be explained by the high red clover proportion, in accordance with the results of Bayat et al. (2010). Concentrations of VFA and lactic acid indicate well-preserved forages with slightly more extensive fermentation for ORG-LG and CON-PR than for ORG-SG and CON-TI.

Effect of Botanical Composition on the Ruminal Biohydrogenation of FA

The increased degree of biohydrogenation of FA with the increased level of initial unsaturation is in accordance with the report of Ferlay et al. (1993). The lower extent of biohydrogenation of C18:3n-3 in the diet containing red clover compared with the grass-based diets is in accordance with the results of Loor et al. (2003), Dewhurst et al. (2003a), and Lee et al. (2008). Lee et al. (2008) also found decreased biohydrogenation of C18:2n-6, and Loor et al. (2003) additionally found decreased biohydrogenation of C18:1 *cis*-9. The decreased ruminal flow of C18:0 in total C18 FA suggests decreased biohydrogenation not only for ORG-SG, but also for ORG-LG compared with CON-PR and CON-TI. Because of the low recoveries of C18 FA in the omasum, the calculation of apparent biohydrogenation according to the method of Wu et al. (1991) resulted in lower values than the other method, but relative differences were similar.

The decreasing effect of red clover on biohydrogenation may be due to an increased rumen passage rate, decreasing the time PUFA resides in the rumen and leading to decreased biohydrogenation and increased bypass of C18:3n-3 (Vanhatalo et al., 2007). However, rumen passage rate was not measured in the present experiment. White clover increases rumen passage rate even more than red clover (Dewhurst et al., 2003a), and the higher white clover proportion in ORG-LG silage

may explain the decreased apparent biohydrogenation of total unsaturated C18 FA compared with CON-PR and CON-TI. Other possible explanations for decreased biohydrogenation when feeding clover silages are related to the effects of secondary plant metabolites (Lee et al., 2007; Cabiddu et al., 2010) or changes in rumen microbe composition (Huws et al., 2010).

Effect of Botanical Composition on the Terminal Step of Ruminal Biohydrogenation

The higher omasal flow of C18:1 *trans* FA for ORG-LG compared with ORG-SG may have been caused by the higher intake of the precursors C18:2n-6 and C18:3n-3. For ORG-SG, the higher omasal flows of C18:1 *trans* FA could not be explained by intakes of C18:2n-6 and C18:3n-3. However, the higher omasal proportions of C18:1 *trans* FA in the total C18 FA for ORG-SG and ORG-LG compared with CON-PR and CON-TI indicates an inhibition of the terminal biohydrogenation step from C18:1 *trans*-11 to C18:0, leading to an accumulation of C18:1 *trans* FA (Lock and Garnsworthy, 2003; Lourenço et al., 2008). Inhibition of the terminal biohydrogenation step may be related to changes in the rumen microbe composition or PUFA intake (Maia et al., 2007). Effects of forage botanical composition on omasal flows of C18:1 *trans* FA are smaller than the effects of concentrate level or oil supplementation (Loor et al., 2004; Lee et al., 2008). However, according to Lee et al. (2008), red clover silage increases the duodenal flow of C18:1 *trans* FA compared with perennial ryegrass silage, and the flow of C18:1 *trans*-10 increases more than the flow of C18:1 *trans*-11.

In addition, the higher milk fat proportion of C18:2 *trans*-10, *cis*-12 for ORG than for CON indicates changes in rumen microbes associated with the lower milk fat proportion of C16:0 (Shingfield and Grinari, 2007). However, the milk fat proportion of C18:2 *trans*-10, *cis*-12 for ORG was low compared with those of fish

Table 5. Intake, omasal flow, and apparent biohydrogenation of C18 FA and C20:1 *cis*-9 in cows fed the experimental silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass ley (CON-PR), or conventionally managed timothy ley (CON-TI; n = 4)

Item	Treatment				SED ¹	Silage	P-value		
	ORG-SG	ORG-LG	CON-PR	CON-TI			ORG-SG vs. ORG-LG ²	CON-PR vs. CON-TI ²	ORG vs. CON ²
Intake, g/d									
C18:0	6.1 ^a	6.1 ^a	6.4 ^a	5.0 ^b	0.21	0.003	NS	<0.001	0.03
C18:1 <i>cis</i> -9	24.0 ^b	28.0 ^a	22.6 ^b	22.0 ^b	0.83	0.001	0.003	NS	<0.001
C18:2n-6	95.8 ^b	112.0 ^a	90.5 ^b	88.1 ^b	4.24	0.005	0.009	NS	0.003
C18:3n-3	90.3 ^b	124.6 ^a	126.8 ^a	85.2 ^b	9.43	0.008	0.01	0.005	NS
Total unsaturated C18 FA	212.9 ^b	268.1 ^a	242.9 ^{ab}	198.1 ^b	13.47	0.008	0.006	0.02	0.08
Total C18 FA	218.9 ^b	274.3 ^a	249.3 ^{ab}	203.1 ^b	16.84	0.008	0.007	0.01	0.08
C20:1 <i>cis</i> -9	1.0	1.1	1.0	0.9	0.07	NS	NS	NS	NS
Omasal flow, g/d									
C18:0	119.2 ^b	153.0 ^a	144.2 ^{ab}	130.6 ^{ab}	8.77	0.03	0.009	NS	NS
C18:1 <i>cis</i> -9	8.2 ^{ab}	9.7 ^a	6.5 ^b	6.3 ^b	0.78	0.01	0.10	NS	0.003
C18:1 <i>trans</i> FA	19.8 ^b	25.5 ^a	12.4 ^c	11.1 ^c	0.84	<0.001	<0.001	NS	<0.001
C18:2 <i>cis</i> -9, <i>trans</i> -11	1.1	1.9	1.5	1.3	0.32	NS	0.05	NS	NS
C18:2n-6	14.9	14.8	10.9	10.8	1.73	NS	NS	NS	0.05
C18:3n-3	6.0 ^a	5.5 ^{ab}	5.2 ^{ab}	3.5 ^b	0.69	0.05	NS	0.05	0.03
Total unsaturated C18 FA	48.8 ^{ab}	58.3 ^a	37.2 ^{bc}	33.5 ^c	3.45	0.001	0.03	NS	<0.001
Total C18 FA	168.0 ^b	211.3 ^a	181.4 ^{ab}	164.1 ^b	10.58	0.02	0.006	NS	0.06
C20:1 <i>cis</i> -9	0.3	0.5	0.5	0.4	0.11	NS	NS	NS	NS
Omasal flow in total C18 FA, g/kg									
C18:0	706 ^b	726 ^b	794 ^a	792 ^a	14.1	0.001	NS	NS	<0.001
C18:1 <i>trans</i> FA	119 ^a	122 ^a	69 ^b	70 ^b	5.4	<0.001	NS	NS	<0.001
Apparent biohydrogenation, ³ g/kg									
C18:1 <i>cis</i> -9	654	657	711	713	21.6	0.05	NS	NS	0.01
C18:2n-6	864	869	879	876	13.6	NS	NS	NS	NS
C18:3n-3	932 ^b	956 ^a	959 ^a	958 ^a	4.8	0.004	0.003	NS	0.006
Total unsaturated C18 FA	769 ^b	782 ^b	846 ^a	827 ^a	9.1	<0.001	NS	0.08	<0.001
C20:1 <i>cis</i> -9	647	578	501	625	57.2	NS	NS	0.07	NS
Apparent biohydrogenation in total C18 FA, ⁴ g/kg									
C18:1 <i>cis</i> -9	545	558	604	642	37.7	NS	NS	NS	0.04
C18:2n-6	822	832	833	848	18.2	NS	NS	NS	NS
C18:3n-3	912 ^b	944 ^a	943 ^a	948 ^a	5.6	0.002	0.001	NS	0.004
Total unsaturated C18 FA	698 ^b	719 ^b	789 ^a	787 ^a	14.4	0.001	NS	NS	<0.001

^{a-c}Means within a row with different superscripts differ (Tukey-Kramer test, $P < 0.05$).¹Standard error of difference.²Contrasts.³ $1,000 - 1,000 \times (\text{omasal flow of FA}/\text{intake of FA})$.⁴ $1,000 - 1,000 \times [(\text{omasal flow of FA}/\text{omasal flow of total C18-FA})/(\text{intake of FA}/\text{intake of total C18-FA})]$ (Wu et al., 1991).

Table 6. Milk fat proportions of selected FA, calculated sums, n-6:n-3 FA ratio, apparent recoveries of FA in the omasum and milk, and apparent de novo synthesis of FA in cows fed the experimental silages from organically managed short-term grassland (ORG-SG), organically managed long-term grassland (ORG-LG), conventionally managed perennial ryegrass ley (CON-PR), or conventionally managed timothy ley (CON-TI; n = 4)

Item	Treatment				SED ¹	P-value			
	ORG-SG	ORG-LG	CON-PR	CON-TI		Silage	ORG-SG vs. ORG-LG ²	CON-PR vs. CON-TI ²	ORG vs. CON ²
FA, g/kg of FAME ³									
C10:0	39.6	38.4	40.0	38.8	0.71	NS	NS	NS	NS
C12:0	47.5	45.3	48.3	46.0	1.05	0.09	0.08	0.07	NS
C14:0	150.4	146.2	147.3	146.7	1.99	NS	0.08	NS	NS
C14:1 <i>cis</i> -9	15.1	13.8	15.1	14.5	0.71	NS	NS	NS	NS
C15:0	13.2 ^a	12.0 ^{ab}	12.3 ^{ab}	11.2 ^b	0.40	0.01	0.03	0.03	0.03
C16:0	351.7 ^b	341.1 ^b	363.8 ^{ab}	378.1 ^a	7.60	0.01	NS	NS	0.004
C16:1 <i>cis</i> -9	15.8	14.9	15.9	16.1	0.38	0.09	0.07	NS	0.06
C18:0	79.3	93.8	85.1	85.6	5.68	NS	0.04	NS	NS
C18:1 <i>cis</i> -9	155.4	160.8	155.8	152.7	4.15	NS	NS	NS	NS
C18:1 <i>cis</i> -11	5.7 ^{ab}	6.5 ^a	5.9 ^a	4.1 ^b	0.45	0.009	0.10	0.007	0.01
C18:1 <i>trans</i> FA	20.0 ^a	22.5 ^a	11.3 ^b	11.3 ^b	1.13	<0.001	0.07	NS	<0.001
C18:2n-6	18.6 ^a	17.2 ^{ab}	14.7 ^b	14.0 ^b	1.10	0.02	NS	NS	0.004
C18:2 <i>cis</i> -9, <i>trans</i> -11	4.7 ^a	5.2 ^a	2.6 ^b	2.4 ^b	0.24	<0.001	NS	NS	<0.001
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.4 ^{ab}	0.5 ^a	0.2 ^b	0.2 ^b	0.08	0.02	NS	NS	0.005
C18:3n-6	0.9	1.6	0.9	0.8	0.44	NS	NS	NS	NS
C18:3n-3	8.8 ^a	6.5 ^{ab}	6.8 ^{ab}	4.9 ^b	0.73	0.01	0.02	0.04	0.01
C18:4n-3	0.3 ^a	0.3 ^a	0.2 ^{ab}	0.1 ^b	0.04	0.010	NS	NS	0.002
C20:0	1.5	1.7	1.5	1.6	0.12	NS	NS	NS	NS
C20:1 <i>cis</i> -9	1.7 ^a	1.4 ^{ab}	1.2 ^{ab}	1.1 ^b	0.17	0.04	0.07	NS	0.02
Calculated proportions and ratio									
Total FA, g/kg of milk	37.0	34.5	35.6	40.9	2.45	NS	NS	0.07	NS
SFA	742.9 ^b	739.7 ^b	760.0 ^a	768.6 ^a	4.91	0.003	NS	NS	<0.001
MUFA	218.6 ^{ab}	224.6 ^a	209.9 ^{ab}	204.9 ^b	4.29	0.01	NS	NS	0.003
PUFA	38.5 ^a	35.7 ^{ab}	30.2 ^{bc}	26.4 ^c	2.18	0.006	NS	NS	0.001
n-6:n-3 FA	20.2 ^b	24.5 ^a	19.8 ^b	25.9 ^a	1.04	0.002	0.006	0.001	NS
Apparent recovery in omasum from feed, g/kg									
C18-FA	720	778	728	817	48.1	NS	NS	NS	NS
Total FA	818	815	763	847	46.2	NS	NS	NS	NS
Apparent recovery in milk from feed, g/kg									
C18:2n-6	122 ^a	95 ^b	103 ^{ab}	104 ^{ab}	6.7	0.03	0.007	NS	NS
C18:3n-3	61 ^a	33 ^b	34 ^b	38 ^b	2.7	<0.001	<0.001	NS	0.001
Apparent recovery in milk from omasum, g/kg									
C18 FA	1,139	930	981	1,107	109.2	NS	0.10	NS	NS
C20 to C24 FA	896	748	767	771	108.2	NS	NS	NS	NS
n-3 FA	717	567	594	580	72.3	NS	0.08	NS	NS
n-6 FA	1,041	861	973	1,007	144.1	NS	NS	NS	NS
Apparent de novo synthesis, ⁴ g/kg of total FA									
C4 to C8	55	57	57	57	2.7	NS	NS	NS	NS
C10 to C16	532 ^b	497 ^b	541 ^a	567 ^a	8.2	<0.001	0.005	0.02	<0.001

^{a-c}Means within a row with different superscripts differ (Tukey-Kramer test, $P < 0.05$).

¹Standard error of the difference.

²Contrasts.

³Fatty acid methyl esters.

⁴Sum of milk FA per day minus sum of FA omasal flow per day divided by sum of milk FA per day.

oil diets, which were found to depress milk fat concentration (Shingfield and Griinari, 2007).

Lourenço et al. (2005) also found incomplete biohydrogenation when replacing silage from intensively managed grassland with silage having a high proportion of dicotyledons from seminatural grassland. In that study, with an increasing proportion of dicotyledon-rich silage, the fat proportion of C18:1 *trans*-11 + *trans*-10 increased in rumen content, but not the proportion of C18:2 *cis*-9,*trans*-11, in accordance with the present study. Also in agreement with the present study, milk fat proportions of both C18:1 *trans*-11 and C18:2 *cis*-9,*trans*-11 increased, but not C18:3n-3.

Khiaosa-Ard et al. (2009) found evidence for inhibition of the terminal biohydrogenation step by extracts containing condensed tannins in vitro. Other secondary plant metabolites with antimicrobial activity (e.g., phenolics and flavonoids) found in plant species, such as garlic (*Allium sativum* L.), rosemary (*Rosmarinus officinalis* L.), and thyme (*Thymus vulgaris* L.; Lai and Roy, 2004), may also change rumen microbe composition.

Effect of Botanical Composition on the Transfer of FA to Milk

Despite the decreased biohydrogenation of C18:3n-3 for ORG-SG, milk fat proportion of C18:3n-3 was no higher than those for ORG-LG and CON-PR, which can be explained by a lower intake of C18:3n-3. This is in contrast to most comparisons of red clover-containing silages with grass silages (Dewhurst et al., 2003b; Höjer et al., 2012).

Higher proportions of the biohydrogenation intermediates C18:1 *trans* FA in milk fat for ORG-SG and ORG-LG reflected the higher omasal flow of this FA. In addition, the milk fat proportion of C18:2 *cis*-9,*trans*-11 was increased in ORG-SG and ORG-LG despite similar omasal flows. This can be explained by endogenously synthesized C18:2 *cis*-9,*trans*-11. The Δ^9 -desaturation of C18:1 *trans*-11 in the mammary gland is the main source of C18:2 *cis*-9,*trans*-11 in milk fat and is therefore correlated with a high rumen outflow of C18:1 *trans*-11 (Griinari et al., 2000), as observed for ORG-SG and ORG-LG. Increased milk fat proportions of C18:2 *cis*-9,*trans*-11 have been associated with dicotyledon-rich silages (Lourenço et al., 2005) or dicotyledon-rich mountain or highland pastures in the Alps (Collomb et al., 2002). The effect of red clover on proportion of C18:2 *cis*-9,*trans*-11 in milk fat is less consistent. Dewhurst et al. (2003b) found a small increase, whereas Al-Mabruk et al. (2004) found a decrease of C18:2 *cis*-9,*trans*-11 in milk fat compared with that in grass silages.

The lower milk fat proportion of C16:0 for the ORG silages than the CON silages may have been caused by lower de novo synthesis, but shorter SFA were not affected. Lourenço et al. (2008) concluded that feeding botanically diverse forages decreases the proportion of de novo-synthesized FA compared with feeding grass-based forages, a finding reaffirmed by the present experiment. In contrast, red clover has not consistently been related to changes in de novo synthesis of FA. Dewhurst et al. (2003b) found decreased C12:0, C14:0, and C16:0 with an increased red clover proportion in silage, but Al-Mabruk et al. (2004) found no effect on these FA. Regarding C18 to C24 FA in the omasum apparently recovered in milk, the apparent recovery of C18 FA and n-6 FA in milk relative to omasal flow was high, but it is not clear why C20 FA to C24 FA and n-3 FA had lower apparent recoveries.

Potential Health Effects of Milk Produced on Silages with a High Proportion of Clover or Other Dicotyledons

Decreased milk fat proportions of C16:0 and higher proportions of C18:2 *cis*-9,*trans*-11 and C18:1 *trans*-11, as for ORG relative to CON, may be considered beneficial to human health. This is because C16:0 increases high-density lipoprotein cholesterol (Mensink et al., 2003), whereas increased intake of C18:2 *cis*-9,*trans*-11 may decrease the risk of colorectal cancer (Larsson et al., 2005), and in humans, C18:1 *trans*-11 can be converted to C18:2 *cis*-9,*trans*-11 (Turpeinen et al., 2002). However, the n-6:n-3 ratio was not affected by ORG compared with CON.

CONCLUSIONS

Feeding silages with different botanical compositions affected intake, biohydrogenation, and transfer of unsaturated FA from feed to milk. Ruminal biohydrogenation of C18:3n-3 was decreased by silage containing red clover, and the terminal biohydrogenation step was inhibited by silage containing a high proportion of dicotyledons, leading to a higher outflow of C18:1 *trans* FA compared with those for grass-based silages. In addition, the milk fat proportions of C16:0 were lower for silages with high proportions of dicotyledons. This resulted in milk with high fat proportions of C18:1 *trans* FA and C18:2 *cis*-9,*trans*-11 and reduced proportions of SFA. Thus, feeding dairy cows silage with high proportions of red clover and other dicotyledons, typical in organic dairy production, results in milk with a FA composition regarded as beneficial for human health.

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