Effects of replacement of late-harvested grass silage and barley with early-harvested silage on ruminal digestion efficiency in lactating dairy cows

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ABSTRACT

The objective of this experiment was to quantify the effects of graded replacement of late-harvested grass silage and barley with early-harvested silage on nutrient digestion and rumen fermentation. Four experimental diets were fed to 4 multiparous rumen-cannulated Nordic Red cows in 4 × 4 Latin square design with 21-d periods. Dietary treatments consisted of late-cut grass silage (LS) and rolled barley, which was gradually replaced with early-cut grass silage [ES; 0, 33, 67, and 100% of the forage component (ES + LS) of the diet]. With increased proportion of ES in the diet, the proportion of barley decreased from 47.2 to 26.6% on a dry matter basis. Early- and late-cut silages were harvested at 2-wk intervals (predicted concentrations of metabolizable energy 11.0 and 9.7 MJ/kg of dry matter). The 4 diets were formulated to support the same milk production. Nutrient flows were quantified using omasal sampling technique applying the triple-marker method (Cr, Yb, and indigestible neutral detergent fiber) and ¹⁵N as a microbial marker. Feed intake decreased with graded replacement of LS and barley with ES, but milk production was not influenced by diet. Digestibility of nutrients improved with graded addition of ES in the diet with the greatest difference observed in digestibility of neutral detergent fiber (NDF) and potentially digestible NDF (pdNDF). The results suggested that improved cell wall digestibility with graded level of ES in the diet was partly related to higher intrinsic digestibility of ES than LS, and partly due to negative associative effects with an increased proportion of LS and barley in the diet. Efficiency of microbial N synthesis was not influenced by the diet, but ruminal protein degradability increased with ES in the diet. Rumen fermentation pattern was not affected by the diet despite

large difference in the profile of dietary carbohydrates. Rumen pool size of NDF and pdNDF, and ruminal turnover time of NDF decreased with graded addition of ES in the diet, whereas digestion rate of pdNDF improved. The results of this study indicate that increased CH₄ yield in a parallel production study with graded addition of ES in the diet were more related to greater ruminal and total digestibility of organic matter than to the changes in rumen fermentation pattern.

Key words: silage digestibility, concentrate, nutrient digestion, rumen fermentation

INTRODUCTION

Nutritional strategies to mitigate CH₄ emissions have been intensively sought to identify alternative hydrogen sinks in the rumen. With high-grain diets that are common in feedlot situations with beef cattle, typical CH₄ loss may drop to approximately 3% of gross energy (Johnson and Johnson, 1995), which is much lower than 6 to 7% of gross energy with typical dairy cow diets (e.g., Yan et al., 2000). This suggests that feeding more grain in diets for dairy cows could be an effective strategy to reduce emissions. However, recent data have indicated that the effect of proportion of dietary concentrate supplementation is relatively small within the range of typical dairy cow diets (Ramin and Huhtanen, 2013).

In northern latitudes of Europe, grass silage is the main dietary ingredient and carbohydrate source for dairy cows (Huhtanen et al. 2013). It is well known that early harvested grass silage of high quality can support higher milk yields with lower input of purchased concentrate feed than a silage harvested at a more mature stage (Ferris et al., 2001; Kuoppala et al., 2008; Randby et al., 2012). Additionally, cereal grains can be used directly as human food or more efficiently by monogastric animals, but on the other hand substitution of starch with fiber might contribute to increased CH₄ production by promoting acetate rather than propionate fermentation in the rumen (Moss et

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al., 2000). A companion production study published previously demonstrated that milk production and CH₄ intensity (g of CH₄/kg of ECM) were not influenced, but a greater CH₄ yield (g of CH₄/kg of DMI) was observed when medium-quality late harvested grass silage and rolled barley were replaced by high-quality early harvested grass silage (Cabezas-Garcia et al., 2017). Several digestive mechanisms may contribute to the effects on CH₄ production when different carbohydrate sources are fed to dairy cows, and any nutritional strategy should ideally also strive to reduce potential environmental emissions.

The aim of this experiment was to explain the mechanisms explaining differences in CH₄ emissions when replacing late-harvested grass silage and barley with early-harvested grass silage in the diets to lactating dairy cows. The approach of investigating the effect of grass cut early or late and at the same time reducing concentrate level with early cutting is novel, and will provide insight into the real mechanisms whereby changes in diet composition alter rumen function and nutrient use.

MATERIALS AND METHODS

All animals were registered and cared for according to guidelines approved by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority, and the experiment was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Sweden. The present experiment was conducted in parallel with a production study, which evaluated production responses and CH₄ emissions in 16 lactating cows fed the same experimental diets (Cabezas-Garcia et al., 2017).

Cows, Diets, and Experimental Design

Four multiparous Nordic Red cows, averaging (mean $\pm$ SD) 676 $\pm$ 79 kg of BW, 90 $\pm$ 19.1 DIM, and producing 31 $\pm$ 6.3 kg of milk/d at the beginning of the study were used in a balanced 4 $\times$ 4 Latin-square design. The experimental periods lasted for 21 d, and were divided into 14 d of adaptation, and 7 d of data recording and sampling. All cows were fitted with 10-cm ruminal cannulas (Bar Diamond Inc., Parma, ID). The cows were housed in tie stalls bedded with sawdust. They had free access to water and salt blocks during the whole experiment, and were offered feed twice daily at 0600 and 1800 h. Orts were recorded once daily and feeding rate adjusted to yield orts of approximately 10% of the intake except during the 4 d of sampling from the omasum, when it was restricted to 95% of ad libitum

intake to minimize between- and within-day variations in intake due to intensive sampling. Total DMI and milk production were recorded daily. Milking was performed twice daily at 0700 and 1600 h.

Two grass silages were harvested at 2-wk interval (June 10 and 24, 2013) using a disc mower conditioner (GMT 3605 FlexP, JF-Stoll A/S, Sønderborg, Denmark) and a precision-chop forage wagon (ES 5000 MetaQ Protec, JF-Stoll A/S). The herbage was harvested as a primary growth of a third-year ley dominated by timothy grass (*Phleum pratense*) with some red clover (*Trifolium pratense*) in Umeå, Sweden (63°45' N, 20°17'). The crops were wilted to an approximate DM concentration of 300 g/kg and ensiled in bunker silos using a commercial acid-based additive (ProMyr XR 630, Perstop, Sweden) provided at a rate of 3.5 L/t.

The experimental diets were formulated to meet the ME and MP requirements for production of 35 kg/d of ECM within the constraints of intake potential (see Cabezas-Garcia et al., 2017). The dietary treatments in the study comprised gradually replacing late-cut silage (**LS**) and rolled barley with 3 incremental levels of early-cut silage (**ES**). A mixture of LS and barley were gradually replaced with ES (0, 33, 67, and 100% of the forage component of diet) to obtain 4 diets defined as follows: late-cut (**L**), late-early (**LE**), early-late (**EL**), and early-cut (**E**) silage. With increasing proportion of ES in the diet, proportion of barley decreased from 47.2 to 26.6% on a DM basis. The changes in the proportion of total forage, barley, and proportions of LS and ES within the forage component were all linear. Heat-treated solvent-extracted canola meal containing low levels of glucosinolates and erucic acid (ExPro-00SF from AarhusKarlshamn Ltd., Malmö, Sweden) was used as a protein supplement. Because the cows in the production study (Cabezas-Garcia et al., 2017) received a compound feed (Solid 220; Lantmännen Lantbruk AB, Malmö, Sweden) while visiting the GreenFeed system (GreenFeed system, C-Lock Inc., Rapid City, SD) for recording CH₄ emissions, the rumen-cannulated cows were offered 1 kg of DM/d of the same compound feed to have a similar diet composition in both studies. The diets were fed as TMR. The DM concentration of the grass silages was determined twice a week and once a week for the concentrate ingredients for adjustment of diet composition. The formulation of the experimental diets is shown in Table 1.

Sampling Procedures

Total DMI and milk yield were recorded daily, but only data from d 14 to 21 were used for statistical analysis. Milk yield was recorded twice daily with gravimetric milk recorders (SAC, S.A. Christensen and

Co Ltd., Kolding, Denmark). Samples (~20 mL) for milk composition analysis were collected at morning and evening milking on 4 consecutive days from the afternoon of d 18 until the morning of d 20 in every period. Two rumen evacuations were conducted at 4 h after (d 12) and 1 h (d 14) before the morning feeding, respectively, to give an estimate of the rumen pool size. An arithmetic mean of the 2 evacuations was used in further calculations. During the evacuations, particulate and fluid matters were separated, weights were recorded, and a rumen sample was reconstituted based on the recorded proportions and stored at -20°C until oven drying and analysis.

Continuous infusion of the flow markers Yb-acetate (3.0 g/d of Yb) and CrEDTA (3.0 g/d of Cr) were started on d 15 in each period. The CrEDTA was prepared as described by Binnerts et al. (1968). Marker infusions were continued until the last digesta sample was collected on d 21. Also from d 15, $(^{15}\text{NH}_4)_2\text{SO}_4$ with 10%

atom excess (Sigma Aldrich Isotec, Miamisburg, OH), providing 200 mg/d of ^{15}N was infused using a peristaltic pump (ISMATEC Reglo Digital; IDEX Health and Science SA, Glattbrugg, Switzerland) assembled with individual lines providing the flow marker infused directly into the rumen of each cow. Infusion started at least 60 h before the first digesta sample collection in all periods to allow uniform distribution of ^{15}N from incorporation of $^{15}\text{NH}_3$ in the ruminal microbial pools (Broderick and Merchen, 1992). Before starting infusion of ^{15}N , a sample of ruminal digesta was taken from each cow in every period and stored at -20°C for later determination of ^{15}N background.

The omasal sampling technique (Huhtanen et al., 1997), as modified by Ahvenjärvi et al. (2000), was used to collect 12 digesta samples from the omasum on d 17 to 20 with 4-h interval between the 3 sampling occasions each day to cover sampling hourly during a complete 12-h feed cycle. On each day the sampling

Table 1. Ingredient and diet composition

Item	Diet ¹				Ingredient ²				
	L	LE	EL	E	LS ³	ES ³	Barley	Canola meal	Concentrate mix ⁴
Ingredient, g/kg of DM									
LS	421	329	189	0					
ES	0	164	378	641					
Barley	472	404	335	266					
Canola meal expeller	97	93	89	84					
NaCl	3.2	3.2	3.1	3.1					
CaCO ₃	6.6	6.2	5.8	5.3					
Nutrient composition, ⁵ g/kg of DM									
DM, g/kg	449	432	412	378	327	293	902	907	880
Ash	70.3	69.7	69.9	69.2	64.7	64.3	60.8	58.1	63.3
CP	156	162	170	180	139	179	122	390	202
NDF	324	352	382	410	605	575	188	367	197
Indigestible NDF	94.7	92.6	88.4	81.9	149	92.8	43.9	143	64.9
pdNDF ⁶	229	259	294	328	456	482	144	224	132
Starch	251	219	185	151	NA ⁷	NA	503	16.0	357
Feeding value									
ME, ⁸ MJ/kg of DM	11.6	11.6	11.6	11.7	11.0	9.7	13.2	12.3	12.9
DOM _{Maint} , ⁹ g/kg of DM	747	745	744	747	635	707	821	696	752
MP, g/kg of DM	95.2	95.4	95.7	96.7	85.7	73.9	97.8	171	120
PBV, ¹⁰ g/kg of DM	13.7	20.2	27.7	36.2	51.7	29.1	-20.2	117	21.0

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Forages: LS = late-cut silage; ES = early-cut silage; barley = rolled barley.

³Fermentation quality of LS and ES, respectively, pH = 3.89, 3.67; lactic acid = 86.1, 99.6; acetic acid = 12.2, 21.6; propionic acid = 1.48, 1.06; and butyric acid = 0.23, 0.27 g/kg of DM, and $\text{NH}_3\text{-N}$ = 23.1, 45 g/kg of N.

⁴Concentrate mix = Solid 220 (Lantmännen, Malmö, Sweden). In addition to the TMR diets, the cows received 1 kg of concentrate mixture on a DM basis.

⁵Nutrient composition of TMR + 1 kg on a DM basis of concentrate mix.

⁶pdNDF = potential digestible NDF.

⁷NA = not analyzed.

⁸Based on coefficients from feed tables (LUKE, 2016).

⁹DOM_{Maint} = digestible OM for silages at maintenance according to Huhtanen et al. (2013) and concentrate feed from tabulated values (LUKE, 2016).

¹⁰PBV = protein balance in the rumen.

occasions were moved 1 h later than on previous day. At each sampling, a 650-mL digesta was collected and divided in 2 subsamples as follows: 400 mL from every time point was pooled and held at -20°C as they were collected to yield a 4.8-L omasal composite sample, 250 mL at 3 collections were pooled and kept in a refrigerator to yield a 750-mL digesta sample, one from each of the 4 digesta collection days. Fecal spot samples (approximately 300 g), were collected within the same 4 d and time points as the omasal digesta samples. Fecal grab samples were pooled within cow during collection in each period and total-tract digestibility of the diets was determined by using indigestible NDF (iNDF; Huhtanen et al., 1994) as a marker. Composite fecal samples per cow/period were obtained at the end of each feces collection period and oven-dried at 60°C for 48 h. From the 4-d omasal composite samples from all individual cows collected during all periods, 3 digesta phases were separated: large particles, small particles, and a fluid phase, and prepared for analysis according to the procedures described by Krizsan et al. (2010).

At the end of each of the four 12-h sampling days bacteria were isolated from the 4 daily composites made from digesta collected from the omasum of each cow with slight modifications of the method described by Krizsan et al. (2010). The supernatants from the $1,000 \times g$ centrifugations (10 min at 5°C) that aimed to separate liquid- and particle-associated bacteria from feed particles were combined to give one bacterial sample. A pooled bacterial isolate was thereafter separated and cleaned by high-speed centrifugation as described by Fatehi et al. (2015). The resulting bacterial pellets were stored at -20°C until freeze-dried. Bacterial composites were made by pooling equal amounts of sample DM from the 3 sampling days.

Samples of rumen fluid were collected on d 21 at 1.5-h intervals starting before the morning feeding. The rumen fluid was taken from 3 locations in the mid-ventral sac of the rumen of each cow. Samples were transferred to a pre-warmed thermos flasks previously flushed with CO_2 using a metal filter probe connected to a light vacuum at 0 (just before feeding), 1.5, 3, 4.5, 6, 7.5, 9, and 10.5 h after feeding. Samples were strained through 2 layers of cheesecloth, followed by immediate measurement of pH, and collection in tubes of 5 mL for later analysis of fermentation parameters (2.5 mL each). Two tubes containing 0.5 mL of 25% metaphosphoric acid and 2 tubes with 0.25 mL of 2 N sulfuric acid were previously prepared for analysis of VFA and $\text{NH}_3\text{-N}$ concentrations, respectively. All sample tubes were stored at -20°C . Rumenal fluid samples were later thawed, composited on volume basis per cow/period, and analyzed for VFA. Individual rumen fluid samples from different time points were analyzed for $\text{NH}_3\text{-N}$.

In Situ Determination of iNDF and Chemical Analysis

Concentration of iNDF in all feeds, particulate fractions of digesta samples, and fecal samples was determined by 288-h in situ incubation (Huhtanen et al., 1994) according to the procedure described by Krizsan et al. (2015). Indigestible NDF was expressed exclusive of residual ash. Incubations were performed in 3 ruminally fistulated cows fed a TMR of 600 g/kg of DM of grass silage and 400 g/kg of DM of a commercial concentrate mix (Solid 220, Lantmännen, Malmö, Sweden).

The dried individual fecal samples and digesta phases were further analyzed for DM, Yb, and Cr. Feed samples were analyzed for DM. The dried individual feed, fecal, and reconstituted true digesta samples were further analyzed for OM, NDF, N, NAN, and ^{15}N . Residual moisture of all samples was determined by oven drying for 16 h at 105°C . Ash concentration was determined by ignition of the dried sample at 500°C for 4 h (AOAC International, 2012).

Neutral detergent fiber analysis (Van Soest et al., 1991) was performed with a heat-stable α -amylase and sodium sulfite using the filter bag technique in an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Ash was determined in the bag residues and NDF expressed free of residual ash. The concentration of CP in feeds was analyzed by AOAC International (2012) method 990.03 and in feces according to the method proposed by Watson et al. (2003).

Abundance of ^{15}N and concentrations of NAN were analyzed with a Flash 2000 Organic Elemental Analyzer interfaced with an Isotope Ratio Mass Spectrometer Delta V Advantage (Thermo Fisher Scientific Inc., Waltham, MA). Samples were prepared and ammonia volatilized as described by Krizsan et al. (2010). Samples were analyzed in duplicate and samples with similar ^{15}N enrichment were analyzed as groups to avoid ^{15}N carryover between samples. Individual digesta phases and fecal samples were analyzed for Yb and Cr using inductively coupled plasma optical emission spectroscopy on a Perkin-Elmer Optima 7300 DV (Perkin Elmer, Shelton, CT). Samples of approximately 1.5 g of DM were added to 10 mL of a solution containing 65% wt/vol of purified HNO_3 . Samples were boiled in Kjeltex tubes in a block digester system (Tecator Digestion Auto; Foss Analytical A/S, Hillerød, Denmark) with stepwise increasing temperature: 1 h at 60°C , 1 h at 100°C , and finally 4 h at 125°C . After 2 h at 125°C , an additional 5 mL of the HNO_3 solution was added. Samples were diluted to a final volume of 50 mL with distilled water after cooling. Composite bacterial samples and background samples of ruminal digesta, from each cow in every period, were analyzed for NAN

and ^{15}N as described for fecal and digesta samples. Additionally, DM and ash contents were determined in the composite bacterial samples as described above.

Individual VFA concentrations in rumen fluid samples were determined using a Waters Alliance 2795 HPLC system with Waters 2414 RI detector (Waters Corporation, Milford, MA) as described by Ericson and André (2010), and NH_3 according to the method provided by the Seal Analytical (Method nr G-102-93 multitest MT7) on an AutoAnalyzer 3 (Seal Analytical, Mequon, WI).

Frozen silage samples were thawed and pressed, and pH was measured with a pH meter (Metrohm, Herisau, Switzerland). Ammonium-N was analyzed by direct distillation after adding MgO using a Kjeltec 2100 Distillation Unit (Foss Analytical A/S). In addition, VFA and lactic acid were analyzed according to Ericson and André (2010). Concentrations of fat, protein, urea, and lactose in milk were measured by infrared spectroscopy (MilkoScan FT120, Foss Analytical A/S), and analytical values for each individual milking were weighted according to yield at each milking to provide a representative value.

Calculations

In vivo OM digestibility (**OMD**) of silages at a maintenance level of feeding was estimated from iNDF and NDF according to the equation of Huhtanen et al. (2013) and given related to silage DM concentration. Energy-corrected milk was calculated according to Sjaunja et al. (1990). Feed efficiency was calculated from ECM yield and DMI, and milk N efficiency from the milk N yield (protein yield in g/d/6.38) divided by the N intake. Production of CH_4 per mole of VFA (CH_4VFA) was calculated based on VFA stoichiometry according to the equation by Wolin (1960):

$$\text{CH}_4\text{VFA} (\text{mmol/mol VFA}) = 0.5 \times C_2 - 0.25 \times C_3 + 0.5 \times C_4, \quad [1]$$

where C_2 , C_3 , and C_4 are molar proportions (mmol/mol) of acetate, propionate, and butyrate, respectively.

Total-tract digestibility was calculated from the concentration of iNDF related to the relevant nutrient components in the diet and in the fecal samples (the equation for OMD is given as an example; Equation [2]).

$$\text{OMD} = 1 - (\text{iNDF in diet DM} / \text{iNDF in fecal DM}) \times (\text{OM in fecal DM} / \text{OM in diet DM}), \quad [2]$$

where OMD is the total-tract apparent digestibility of OM in grams per kilogram. Metabolic fecal OM was determined as fecal neutral detergent solubles (**NDS**) output per kilogram of DMI, where neutral detergent solubles were determined by subtraction of NDF from OM concentration in feces.

Calculation of omasal flow of nutrients was based on triple-marker method (France and Siddons, 1986), which requires 3 markers and 3 physically separated digesta phases. Indigestible NDF, which is mainly associated with the large particle fraction (Ahvenjärvi et al., 2003), Yb concentrated in the small particle digesta phase, and Cr following the fluid phase (Downes and McDonald, 1964) were used in combination to calculate digesta flow. Proportions of each digesta phase in representative true digesta sample were solved by matrix calculation based on marker concentrations in the digesta phases and daily marker doses recovered from feces according to Armentano and Russell (1985). To calculate true rumen digestibility of OM flow of OM was corrected for VFA flow according to the equation of Huhtanen et al. (2010), and flows of OM and NAN were corrected for microbial OM and NAN flows, respectively. Total NAN flow was assumed to be composed of microbial NAN and nonammonia nonmicrobial N. Microbial NAN flows were calculated from ^{15}N enrichment above the background:

$$\text{microbial NAN flow (g/d)} = \text{NAN flow (g/d)} \times (\text{TD } ^{15}\text{N APE} / \text{bacterial } ^{15}\text{N APE}), \quad [3]$$

where $\text{TD } ^{15}\text{N APE}$ = ^{15}N enrichment of the reconstituted true digesta sample above background and bacterial $^{15}\text{N APE}$ is ^{15}N enrichment of microbial sample above background, and APE is the atom percent excess. The nonammonia nonmicrobial N flow was calculated as the difference between total NAN flow and total microbial NAN flow. Recovery of ^{15}N at the duodenum was calculated as excess ^{15}N flow to the omasum in mg/d divided by ^{15}N infused in mg/d.

Digestion kinetic variables were calculated based on the compartmental flux method described by Ellis et al. (1994). Passage rate (k_p) of iNDF was determined as

$$k_p (\%/h) = 100 \times \text{iNDF fecal output (kg/h)} / \text{rumen pool of iNDF (kg)}. \quad [4]$$

Calculation of k_p of potentially digestible NDF (**pdNDF**) was determined using the same principle as in Equation [4]. Rate of digestion (k_d) of pdNDF was calculated by subtracting k_p from rate of intake of

pdNDF (calculated from pdNDF intake/h divided by rumen pdNDF pool).

Turnover time of NDF was calculated based on the same assumption:

$$\text{NDF turnover (h)} = 24 \text{ h} \times \frac{\text{rumen pool of NDF (kg)}}{\text{intake of NDF (kg)}} \quad [5]$$

Statistical Analysis

Data were analyzed for treatment effects using the MIXED procedure of SAS (SAS Inc. 2002–2003, release 9.2, SAS Institute Inc., Cary, NC) by applying a model correcting for the effect of cow, period, and diet:

$$Y_{ijk} = \mu + C_i + P_j + D_k + \varepsilon_{ijk}, \quad [6]$$

where Y_{ijk} is the dependent variable and μ is the mean for all observations, C_i is the random effect of cow i , P_j is the effect of period j , D_k is the effect of diet k , and $\varepsilon_{ijk} \sim N(0, \sigma_e^2)$ is the random residual error, with an expected mean of 0 and σ_e^2 as the constant variance. Polynomial contrasts were included for evaluation of linear and quadratic responses to dietary replacement of LS and barley with ES. Data on pH and NH_3 measurements were evaluated in a repeated measurements model using the Toeplitz function in SAS MIXED procedures (cow within period was used as subject). The model accounted for effects of cow, period, diet, time, and interactions between diet and time.

RESULTS

Diet Composition and Production

Ingredient and diet composition are given in Table 1. The 2 grass silages differed in digestibility, as anticipated, and were well preserved in terms of silage fermentation parameters (pH <4.0), low concentrations of butyric acid (<1 g/kg of DM) and ammonia N (<60 g/kg of total N). The late-cut silage had a lower CP concentration and higher NDF, especially iNDF, concentration than early-cut silage as expected due to differences in forage maturity.

Replacement of barley and LS with ES was reflected in decreased starch and increased pdNDF concentration in the diets. Additionally, due to differences in silage CP concentration between LS and ES, dietary CP concentration increased with proportion of ES in the diet. Dry matter intake decreased ($P = 0.02$), but both milk yield and composition were not influenced by the graded addition of ES in the diet (Table 2). Quadratic responses ($P < 0.01$) were detected in milk protein and MUN concentrations with the highest value of milk protein observed for diet L and the lowest MUN for diet LE. There was a linear trend ($P = 0.08$) for improved feed efficiency with increased proportion of ES in the diet.

Rumen Fermentation

Effects of graded addition of ES in the diet on rumen fermentation pattern were minor except for linear

Table 2. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage (ES) on DMI and milk production

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
DMI, kg/d	20.2	19.2	19.1	17.9	0.83	0.02	0.83
Milk, kg/d	22.1	21.3	22.8	22.4	2.03	0.46	0.79
ECM, kg/d	23.5	21.6	22.8	23.0	2.31	0.93	0.31
Composition							
Fat, g/kg	42.4	39.8	39.3	40.6	1.87	0.38	0.22
Protein, g/kg	37.4	36.1	35.7	36.0	0.80	<0.01	<0.01
Lactose, g/kg	46.0	45.6	45.9	46.1	0.95	0.69	0.44
MUN, mg/dL	9.8	8.5	10.4	10.9	0.22	<0.01	<0.01
Yield, g/d							
Fat	948	856	897	916	107.8	0.81	0.30
Protein	828	760	801	801	59.5	0.70	0.19
Lactose	1,021	983	1,050	1,037	106.2	0.40	0.69
Efficiency							
ECM/DMI, kg/kg	1.16	1.11	1.18	1.31	0.11	0.08	0.16
Milk N/N intake, g/kg	266	245	247	256	15.9	0.54	0.17
BW, kg	664	667	663	651	44.4	0.39	0.50

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

Table 3. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage (ES) on rumen fermentation

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
pH	6.04	6.15	6.05	6.15	0.028	0.07	0.67
Ammonia N, mg/dL	6.1	8.0	8.8	8.8	1.06	0.07	0.39
Total VFA, mmol/L	109	103	108	103	3.5	0.27	0.71
Molar proportion, mmol/mol							
Acetate (C ₂)	684	688	686	687	6.0	0.82	0.80
Propionate (C ₃)	167	170	181	178	5.9	0.19	0.61
Butyrate (C ₄)	106	98	94	98	4.8	0.14	0.20
Isovalerate	30.5	30.6	26.8	27.7	0.76	0.01	0.63
Valerate	12.5	12.8	11.4	10.1	0.72	0.03	0.28
VFA ratio							
C ₂ /C ₃	4.10	4.06	3.81	3.87	0.163	0.25	0.79
(C ₂ + C ₄)/C ₃	4.73	4.65	4.34	4.42	0.191	0.19	0.68
CH ₄ VFA ³	369	367	359	362	5.0	0.22	0.60

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

³CH₄VFA = $0.5 \times C_2 - 0.25 \times C_3 + 0.5 \times C_4$, where C₂, C₃, and C₄ are molar proportions (mmol/mol) of acetate, propionate, and butyrate, respectively (Wolin, 1960).

decreases ($P \leq 0.03$) in molar proportions of isovalerate and valerate, a trend for increased pH ($P = 0.07$), and a trend for increased rumen ammonia-N concentration ($P = 0.07$) with increased proportion of ES in the diet (Table 3).

Ruminal and Total Digestibility

Apparent total-tract digestibility of nutrients increased linearly, except that of NDS, as the proportion of ES in the diet increased ($P \leq 0.01$; Table 4). The

greatest difference between L and E diets was observed in NDF digestibility (148 g/kg) accounting for most of the differences in OM digestibility. The proportion of digested NDF of digested OM increased linearly ($P < 0.01$) from 280 to 416 g/kg in response to increasing proportion of ES in the diet.

Increased proportion of ES in the diet linearly decreased ($P \leq 0.02$) intake, omasal flow, and fecal output of OM (Table 5). Both apparent ($P = 0.04$) and true ($P < 0.01$) ruminal OM digestibility increased with graded addition of ES in the diet. On average, apparent rumen

Table 4. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage (ES) on apparent total-tract digestibility of the diet

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
Digestibility, g/kg							
DM	672	666	681	701	5.5	<0.01	0.06
OM	677	674	687	708	5.2	<0.01	0.06
CP	654	640	683	695	10.2	0.01	0.26
NDF	483	500	577	631	8.0	<0.01	0.06
pdNDF ³	651	655	731	771	11.8	<0.01	0.18
NDS ⁴	801	796	772	772	9.4	0.04	0.80
MFOM, ⁵ g/kg of DMI	117	112	119	117	4.9	0.74	0.85
DNDF/DOM, ⁶ g/kg	280	309	372	416	7.7	<0.01	0.37

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

³pdNDF = potentially digestible NDF calculated as NDF – indigestible NDF.

⁴NDS = neutral detergent solubles (OM – NDF).

⁵MFOM = metabolic fecal OM; fecal NDS output per kilogram of DMI.

⁶DNDF/DOM = proportion of digested NDF of digested OM.

Table 5. Effects of graded replacement of late-cut silage and barley with early-cut silage (ES) on OM and fiber digestion

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
OM, kg/d							
Intake	18.8	17.9	17.8	16.6	0.76	0.02	0.83
Flow into omasum	9.4	9.4	7.9	7.7	0.47	<0.01	0.78
Fecal output	6.1	5.9	5.6	4.9	0.25	<0.01	0.08
NDF, kg/d							
Intake	7.4	7.4	7.8	7.7	0.30	0.12	0.62
Flow into omasum	4.3	4.0	3.9	3.2	0.11	<0.01	0.04
Fecal output	3.8	3.7	3.3	2.9	0.14	<0.01	0.15
OM digestibility, g/kg							
Apparent rumen ³	491	466	546	530	17.0	0.04	0.78
True rumen ⁴	700	710	738	758	9.2	<0.01	0.58
Apparent total	677	674	687	708	5.2	<0.01	0.06
Fiber digestibility, g/kg							
NDF rumen	419	460	498	583	12.1	<0.01	0.05
pdNDF ⁵ rumen	564	602	631	714	14.7	<0.01	0.07
NDF total	483	500	577	631	8.0	<0.01	0.06
pdNDF total	651	655	731	771	11.8	<0.01	0.18
NDF rumen/total	0.87	0.92	0.86	0.93	0.029	0.35	0.82

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

³Organic matter apparently digested in the rumen (flow of OM was corrected for flow of VFA).

⁴Organic matter truly digested in the rumen (flow of OM was corrected for microbial OM flow).

⁵pdNDF = potentially digestible NDF calculated as NDF – indigestible NDF.

OM digestibility accounted proportionally for 0.723 g of total OM digestibility but no difference was detected among treatments. There were no differences in NDF intake among the diets ($P = 0.12$), but NDF flow into the omasum and fecal output decreased linearly ($P < 0.01$) with increasing inclusion of ES in the diet (Table 5). Increasing proportion of ES in the diet linearly increased ($P < 0.01$) both ruminal and total digestibility of NDF and pdNDF.

Nitrogen Metabolism

No difference was observed between treatments in N intake, flow of N fractions into the omasum, or fecal N output ($P \geq 0.11$). However, total N flow into the omasum tended to decrease ($P = 0.08$) as proportion of ES in the diet increased (Table 6). Ruminal N degradability and total-tract N digestibility linearly increased ($P \leq 0.04$) with increased proportion of ES in the diet. Decreased ¹⁵N enrichment ($P = 0.03$) of rumen bacteria was observed with increasing proportion of ES in the diet.

Rumen Pool Sizes and Digestion Kinetics

Increased proportion of ES in the diet resulted in linear decreases ($P = 0.03$) in both NDF and pdNDF

pool sizes, and pool sizes of DM and OM tended ($P \leq 0.10$) to decrease (Table 7). Ruminal turnover time of NDF decreased linearly ($P < 0.01$) from 26.2 h with the L diet to 20.4 h with the E diet. No difference was observed in passage rate of iNDF and pdNDF, but intake and digestion rate of pdNDF linearly increased ($P < 0.01$) as proportion of ES in the diet increased.

DISCUSSION

Diet Composition and Production

A production study with the same experimental diets was conducted in parallel with the present study and demonstrated that improvements in forage quality can reduce concentrate supplementation without compromising either milk production or CH₄ intensity (Cabezas-Garcia et al., 2017). The purpose of the current study was to investigate effects of the same diets on digestion physiology to understand possible mechanisms involved in responses observed in CH₄ emissions in the parallel production study.

The differences between ES and LS in the concentrations of DOM and ME were as expected, but the values were lower than expected in both silages, probably due to exceptionally warm weather conditions during early summer. The decline with advancing maturity in

Table 6. Effects graded replacement of late-cut silage and barley with early-cut silage (ES) on ruminal N metabolism

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
¹⁵ N enrichment, APE ³							
Bacteria (B)	0.0578	0.0543	0.0545	0.0483	0.00231	0.03	0.57
Large particles (LP)	0.0287	0.0292	0.0290	0.0251	0.00174	0.06	0.09
Small particles (SP)	0.0352	0.0354	0.0341	0.0309	0.00265	0.24	0.51
Fluid	0.0540	0.0516	0.0523	0.0459	0.00219	0.05	0.39
Ratio							
LP:B	0.497	0.537	0.531	0.520	0.02550	0.50	0.26
SP:B	0.609	0.651	0.625	0.642	0.04280	0.72	0.78
Nitrogen, g/d							
Intake	502	499	520	519	24.2	0.39	0.98
Omasal flow							
Total N	504	524	430	455	30.8	0.08	0.93
Microbial N (MN)	309	347	271	300	25.0	0.34	0.84
Feed N ⁴	195	177	159	155	18.4	0.14	0.72
Feces	172	179	163	155	9.3	0.11	0.40
MN, g/kg of OMTDR ⁵	24.3	27.8	21.4	24.4	1.55	0.42	0.89
Ruminal N degradability, ⁶ g/kg	625	659	700	711	27.3	0.04	0.68
N digestibility, g/kg	651	638	685	708	12.8	<0.01	0.22
N digestibility, lower tract, g/kg	659	658	621	659	12.9	0.48	0.23

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

³Atom percent excess.

⁴Includes endogenous N.

⁵OMTDR = organic matter truly digested in the rumen corrected for VFA flow.

⁶Calculated as (N intake – MN flow)/N intake.

Table 7. Effects graded replacement of late-cut silage and barley with early-cut silage (ES) on rumen digesta pool sizes and digestion and passage kinetics of NDF

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
Fresh weight, kg	85.0	84.3	83.4	81.7	5.00	0.36	0.85
DM, g/kg	143	134	131	129	4.5	0.06	0.49
DM, kg	12.2	11.3	11.0	10.4	0.88	0.10	0.80
OM, kg	11.4	10.7	10.4	9.9	0.77	0.09	0.81
N, g	314	297	293	295	28.4	0.56	0.67
NDF, kg	8.07	7.33	7.04	6.56	0.487	0.03	0.75
iNDF, ³ kg	2.29	2.19	2.01	2.03	0.191	0.23	0.71
pdNDF, ⁴ kg	5.78	5.14	5.03	4.52	0.363	0.03	0.83
iNDF:NDF, g/kg	282	301	285	312	16.5	0.34	0.80
NDS, ⁵ kg	3.35	3.33	3.34	3.34	0.308	0.98	0.95
N:NDS, g/kg	94.0	89.2	88.2	88.6	2.38	0.15	0.32
NDF turnover time, h	26.2	23.8	21.9	20.4	1.04	<0.01	0.63
Kinetics of NDF, 1/h							
pdNDF intake rate	0.0396	0.0472	0.0525	0.0593	0.00301	<0.01	0.89
pdNDF passage rate	0.0138	0.0163	0.0138	0.0135	0.00112	0.51	0.25
pdNDF digestion rate	0.0258	0.0309	0.0387	0.0458	0.00224	<0.01	0.68
iNDF passage rate	0.0354	0.0337	0.0351	0.0294	0.00248	0.16	0.42

¹A mixture of late-cut silage and barley was gradually replaced with early-cut silage (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets as follows: L = late-cut silage; LE = late-early silage; EL = early-late silage; E = early-cut silage.

²Lin = linear effect of dietary addition of ES; Quad = quadratic effect of dietary addition of ES.

³iNDF = indigestible NDF.

⁴pdNDF = potentially digestible NDF.

⁵NDS = neutral detergent solubles.

predicted *in vivo* DOM concentration (5.6 g/d) agrees with observed changes in forage digestibility in northern latitudes with advancing maturity as discussed by Cabezas-Garcia et al. (2017). Differences in silage DM concentration and fermentation quality were too small to influence intake and production potential of forages determined by intrinsic forage characteristics.

Intake and production responses were broadly similar to those observed in the production study with greater number of animals and were discussed in detail (Cabezas-Garcia et al., 2017).

Rumen Fermentation

Despite relatively large differences in dietary starch and NDF concentrations, rumen VFA pattern was similar among treatments. The effect of dietary starch concentration on molar proportion of propionate was not significant in a data-analysis based mainly on grass silage-based diets by Sveinbjörnsson et al. (2006). An analysis of a Finnish data set showed a quadratic relationship between dietary starch concentration and molar proportion of propionate with the minimum value at 200 g/kg of DM (Huhtanen et al., 2013). Murphy et al. (2000) found no difference in molar proportion of propionate in cows fed 50 or 70% on DM basis of grain-based concentrates, but the proportion of butyrate increased with greater proportion of concentrates, which is in agreement with studies in growing cattle (Jaakkola and Huhtanen, 1993) and sheep (Moss et al., 1995). This pattern of change in rumen fermentation pattern in ruminants fed more cereal grain- (mainly barley) based concentrates with grass silage can be because increased starch intake tends to increase protozoal population in the rumen (Chamberlain et al., 1985; Jaakkola and Huhtanen, 1993). The meta-analysis of Eugène et al. (2004) indicated that defaunation induced a significant reduction in molar proportion of butyrate in the rumen, whereas molar proportion of propionate increased.

Methane production per mole of VFA calculated according to Wolin (1960) was not influenced by the diet, suggesting that the increased CH₄ yield observed with increased proportion of ES (Cabezas-Garcia et al., 2017) was not related to changes in rumen fermentation pattern. According to Johnson and Johnson (1995), increased concentrate supplementation markedly decreased CH₄ yield, especially with high-concentrate (>90% of diet DM) feedlot diets. However, within typical ranges of dairy cow diets, the effects of proportion of concentrate were relatively small (Ferris et al., 1999). The model of Sauvant and Giger-Reverdin (2009) predicts maximum CH₄ yield with 35% concentrate on a DM basis that agrees with minimum proportion of

propionate at dietary starch concentration of 200 g/kg of DM (Huhtanen et al., 2013). Effects of concentrate supplementation on CH₄ emissions were discussed in more detail by Cabezas-Garcia et al. (2017).

Digestion

Differences in nutrient digestion were similar to those observed in the production study (Cabezas-Garcia et al., 2017). Calculated DOM concentrations at maintenance were similar among diets, whereas in dairy cows depression in digestibility was greater for diets based on increasing proportion of LS and barley. The present results broadly agree with those of Tyrrell and Moe (1975) and Colucci et al. (1989), who reported that effects of increased concentrate feeding on digestibility diminished as feeding level increased. In a meta-analysis by Nousiainen et al. (2009), increased concentrate supplementation had no effect on diet digestibility despite higher intrinsic digestibility of concentrates than forages. On this basis, reduced diet OMD could be expected because of decreasing silage digestibility with increased proportion of barley in the diet.

Reduced OMD with increased LS and barley resulted mainly from the NDF fraction. According to Mertens (1993), differences in cell wall digestion can be attributed to intrinsic factors determining potential digestibility of NDF in ideal rumen conditions and to extrinsic animal factors (e.g., intake level, diet composition) that determine the extent of intrinsic digestibility achieved. In the present study, both factors were involved simultaneously. The decrease in iNDF concentration (from 293 to 200 g/kg of NDF) indicated a greater potential NDF digestibility with increased proportion of ES in the diet. This together with improved digestibility of pdNDF resulted in large differences in NDF digestibility. The difference between E and L diets in digestibility of NDF and pdNDF was similar to that reported by Kuoppala et al. (2010) between ES + 8 kg of concentrate versus LS + 12 kg of concentrate. Reduced pdNDF digestibility with increased LS and barley can partly be due to negative associative effects of increased starch intake on cell wall digestion and partly due to the slower digestion rate of LS compared with ES. In Kuoppala et al. (2010), the adverse effects of delayed harvest and increased concentrate supplementation were additive. Cell wall digestion is influenced by rumen pH, but in the present study there was no difference in rumen pH that could influence NDF digestion. However, according to Mould et al. (1983) rapidly digested carbohydrates can negatively influence cell wall digestion even when rumen pH is optimal (carbohydrate effect). Reduced pdNDF digestibility with increased concentrate supplementation (Kuoppala

et al., 2008) suggest that reduced pdNDF digestibility with increased LS and barley is not entirely due to slower digestion rate of LS compared with ES.

Rumen pool size of NDF decreased with increased proportion of ES in the diet with pdNDF pool, accounting for most of the difference. In other studies (Gasa et al., 1991; Bosch et al., 1992; Rinne et al., 2002), rumen NDF pool increased with advancing maturity of grass silage. Ruminal turnover time of NDF increased with the proportion of LS and barley in the diet. The difference between L and E diets was greater (5.8 h) than those (2.8 and 3.4 h) calculated from the results of Rinne et al. (2002) and Kuoppala et al. (2010). In their studies, the difference in silage iNDF concentration between early- and late-harvested grass silages was approximately the same as in the current study, and concentrate level was fixed. In the study of Murphy et al. (2000), increasing proportion of concentrate in the diet increased calculated ruminal turnover time of NDF by 5 h. This suggests that increased concentrate supplementation has a negative influence on ruminal NDF digestion. Relative decrease in rumen evacuation derived digestion rate of pdNDF was greater in the present study (43%) than the corresponding decreases of 15% in the study of Rinne et al. (2002) and 30% in the study of Kuoppala et al. (2010). A similar difference was also observed in the relationship between dietary iNDF concentration and rumen NDF pool. In the current study, 1 g/kg of DM increase in dietary iNDF concentration increased rumen NDF pool by 104 g, whereas corresponding responses in their studies were 30 and 43 g per g of iNDF/kg of DM. These indirect comparisons suggest that NDF digestibility was influenced not only by dietary factors but also associative effects were involved.

The results of digestion study suggest that higher digestibility contributed to increased CH₄ yield with increased the proportion of ES in the diet. Apparent OMD was on average 60 g/kg lower for L + LE diets than for EL + E diets. Because only apparently fermented OM can be used for VFA and gas production in the rumen, the differences in rumen fermented OM were greater than in total OM digestion, thereby contributing to greater CH₄ yield with increased proportion of ES in the diet observed in production study (Cabezas-Garcia et al., 2017).

Nitrogen Metabolism

Increase in rumen ammonia N concentration with increased ES in the diet was associated with higher dietary CP concentration. Higher CP concentration also increased ruminal N losses. Reduced ¹⁵N enrich-

ment in rumen bacteria and omasal digesta phases with increased ES in the diet was also indicative of ruminal N losses. Efficiency of microbial N synthesis was not influenced by diet. Kuoppala et al. (2010) reported a lower efficiency of microbial N synthesis with diets based on early-harvested primary and regrowth grass silages compared with corresponding late-harvested silages. In the study of Vanhatalo et al. (2009), maturity at harvest of grass silages did not affect microbial N synthesis.

Ruminal protein degradability increased with ES in the diet, but because proportion of concentrate in the diet decreased, the causative factor remains unclear. Reduced intake of heat-treated rapeseed meal that had a low ruminal CP degradability (Krizsan et al., 2017) could have contributed to the lower ruminal CP degradability with increased ES.

Improved apparent N digestibility in the total tract was mainly related to increased N losses from the rumen with increasing ES, whereas digestibility of N entering the omasal canal in the lower tract was not influenced by diet. Microbial cells are H₂ sinks (Czerkawski, 1986), but dietary effects on efficiency of microbial N synthesis were inconsistent and no conclusion about possible contribution of microbial synthesis on increased CH₄ yield with increased ES can be drawn. However, reduced efficiency of microbial synthesis would contribute to increased CH₄ yield with increased ES in the diet as observed in the parallel production study (Cabezas-Garcia et al., 2017) if part of the increased apparent ruminal OM digestibility with increased ES in the diet was due to reduced efficiency of microbial cell synthesis.

CONCLUSIONS

The results of the present study and parallel production study (Cabezas-Garcia et al., 2017) indicate that it was possible to decrease the proportion of concentrate by improving forage quality in terms of animal performance and without increases in CH₄ intensity. Digestibility of nutrients increased with increasing ES and the losses of potentially digestible OM compared with maintenance level were smaller. Rumen pool size of NDF decreased with increasing ES in the diet, suggesting that the contribution of rumen fill in intake regulation was smaller for these diets. The effects of dietary carbohydrate composition (replacement of starch with pdNDF) on rumen fermentation pattern were small and cannot explain higher CH₄ yield observed in a parallel production study with increased ES in the diet. The results of the current study indicate that increased ruminal and total OMD were the main contributors to differences in CH₄ yield.

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