Effects of replacement of late-harvested grass silage and barley with early-harvested silage on milk production and methane emissions

E. H. Cabezas-Garcia,*¹ S. J. Krizsan,* K. J. Shingfield,†² and P. Huhtanen*¹

*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden

†Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth SY23 3EB, United Kingdom

ABSTRACT

This study evaluated the effects of gradual replacement of a mixture of late-cut grass silage (LS) and barley with early-cut grass silage (ES) on milk production, CH₄ emissions, and N utilization in Swedish Red cows. Two grass silages were prepared from the same primary growth of timothy grass sward but harvested 2 wk apart [11.0 and 9.7 MJ of metabolizable energy/kg of dry matter (DM)]. Four diets, fed as a total mixed ration, were formulated to meet the metabolizable energy and protein requirements of 35 kg of energy-corrected milk (ECM) by gradually replacing a mixture of LS and barley with ES (0, 33, 67, and 100% of the forage component of the diet), whereas the proportion of barley decreased from 47.2 to 26.6% of diet DM. Expeller canola meal was used as a protein supplement. Sixteen Swedish Red cows were used in 4 replicated 4 × 4 Latin squares. Cows were offered diets ad libitum and milked twice daily. Each period of 28 d comprised 14 d of diet adaptation followed by 14 d of data collection. Intake and milk yield were recorded daily, and milk samples were collected on d 19 to 21 and d 26 to 28 of each period. Diet digestibility was determined by grab sampling using indigestible neutral detergent fiber as an internal marker. Gas emissions were measured using the Green-Feed system (C-Lock Inc., Rapid City, SD). Dry matter intake (DMI) linearly decreased from 22.6 to 19.3 kg/d as the proportion of ES increased in the diet. The ECM yield did not differ among treatments, but milk protein yield decreased with increasing proportion of ES in the diet. Because of reduced DMI with increasing ES, feed efficiency (ECM/DMI) improved with an increased proportion of ES in the diet. Nitrogen efficiency (milk N/N intake) did not change despite a linear increase in milk urea N concentration from 9.7 (LS alone) to 11.9

mg/dL (ES alone) with graded replacement of LS and barley by ES in the diet. Lower DMI responses in ES diets were partly compensated for by increased organic matter digestibility (656 g/kg of DM for LS alone; 715 g/kg of DM for ES alone) related to improved forage digestibility at early harvesting. Total CH₄ emissions and CH₄ intensity (CH₄/ECM) were not influenced by diet, but CH₄ yield (CH₄/DMI) increased linearly from 19.5 to 23.0 g/kg of DMI with greater inclusion of ES in the diet. In conclusion, replacing LS and barley with ES improved the conversion of feed to milk without increasing CH₄ emissions or compromising N efficiency. **Key words:** concentrate, feed efficiency, grass silage, methane

INTRODUCTION

Compared with other livestock species, ruminants have the unique ability to transform nonedible feedstuffs (e.g., forages) into highly valuable products for human consumption. However, cattle production has been targeted during recent decades for its contribution to greenhouse gas emissions. In the rumen, enteric CH₄ is produced during microbial fermentation of dietary carbohydrates and, quantitatively less importantly, from protein. Volatile fatty acids, CO₂, H₂, and microbial cells are the end products of fermentation (e.g., Van Soest, 1994). Methanogenesis is an important biochemical pathway because it is the main H₂ sink in rumen conditions (Czerkawski, 1986), but it also represents an energetic loss for the cow that ranges, depending on intake and diet composition, from 2 to 12% of gross energy (GE) intake (Johnson and Johnson, 1995). Therefore, potential improvements in reducing enteric CH₄ emissions might improve the feed efficiency of the animals.

Dry matter intake and diet composition are the key factors affecting CH₄ emissions (Hristov et al., 2013; Ramin and Huhtanen, 2013). Increased concentrate feeding promotes milk production and is considered one strategy for reducing CH₄ intensity. Low CH₄ emissions have been observed for feedlot-type diets containing

Received December 12, 2016.

Accepted March 24, 2017.

¹Corresponding authors: edward.cabezas.garcia@slu.se and pekka.huhtanen@slu.se

²Deceased September 11, 2016.

more than 90% of concentrate (Johnson and Johnson, 1995). However, within common ranges used in dairy cow diets, the effect of concentrate proportion on CH₄ emissions was relatively small (Ferris et al., 1999). In a meta-analysis conducted by Ramin and Huhtanen (2013), differences between dietary carbohydrates (NDF vs. NFC) in their effects on CH₄ yield were marginal for diets containing $\leq 75\%$ concentrates on a DM basis. The model of Sauvant and Giger-Reverdin (2009) predicts maximum CH₄ yield at 35% concentrate on a DM basis and moderate decreases of between 35 and 60% of concentrate. High levels of concentrate supplementation can increase the incidence of acidosis and laminitis (Nocek, 1997) and decrease cell wall digestibility, as discussed by Nousiainen et al. (2009).

Grass silage is usually the main component of dairy cow diets in Nordic countries. Maturity at harvest is the major factor influencing the nutritive value of grass silage because of its effect on digestibility. Digestibility is the most important forage factor influencing silage DMI (Huhtanen et al., 2007) and, consequently, nutrient supply. In a literature review by Rinne (2000), the average DMI and milk yield responses reported were 0.16 and 0.32 kg/d per 10 g/kg of DM increase in silage digestible OM (DOM) concentration. Although CH₄ yield generally increases with improved diet digestibility (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), it usually decreases when expressed per unit of digestible energy (e.g., Beever et al., 1988).

Improving forage quality can allow concentrate supplementation to be reduced without compromising milk production. Huhtanen et al. (2013) compiled results of 4 feeding experiments investigating the effects of digestibility of grass silage and level of concentrate supplementation on milk production and found, on average, that 0.81 kg more concentrate DM was required to compensate for 10 g/kg lower DOM concentration in silage DM. These results suggest that it is possible to maintain the same level of milk production by improving forage quality while reducing concentrate input. However, to our knowledge, no data on the effects of this strategy on CH₄ emissions and N efficiency in dairy production have been reported. The main aim of this study was to investigate whether milk production can be maintained without increasing CH₄ intensity (g of CH₄/kg of ECM) by improving dietary forage quality while simultaneously decreasing the use of concentrate. In the present paper, forage quality refers to OM digestibility.

MATERIALS AND METHODS

All animals were registered and cared for according to guidelines approved by the Swedish University of

Agricultural Sciences (Umeå, Sweden) Animal Care and Use Committee and the National Animal Research Authority (Stockholm, Sweden). The experiment was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Sweden.

Experimental Design, Animals, and Management

A production trial was conducted at Rönnebydalen experimental farm of the Swedish University of Agricultural Sciences (63°45' N, 20°17' E). Sixteen Swedish Red dairy cows (12 multiparous and 4 primiparous; mean BW = 635 $\pm$ 76 kg; 79 $\pm$ 14.4 DIM, producing 34 $\pm$ 6.9 kg of milk/d at the start of the experiment) were used in a replicated 4 $\times$ 4 Latin square design trial. The dietary treatments in the study involved gradually replacing late-cut silage (LS) and rolled barley with 3 incremental levels of early-cut silage (ES).

The experimental periods each lasted for 28 d and were divided into 14 d of adaptation and 14 d of data recording and sampling. The cows were assigned to blocks according to parity and milk yield and were randomly allocated to 1 of the 4 treatments within block (square). The cows were housed in an insulated loose-housing barn equipped with an automatic feed intake recording system and were fed a TMR ad libitum with free access to water. The feed components were mixed using a TMR mixer (Nolan A/S, Viborg, Denmark) and then delivered by an automatic feeding wagon to the feed troughs 4 times per day at 0600, 1100, 1500, and 1900 h. The cows were milked twice per day at 0600 and 1500 h.

Feeds and Diet Formulation

Two grass silages of different predicted in vivo digestibility (DOM: 685 and 607 g/kg of DM) were harvested 2 wk apart (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). Silages were harvested from the primary growth of a third-year ley dominated by timothy grass (*Phleum pratense*), with some red clover (*Trifolium pratense*). The fields were fertilized with 70 kg of N/ha in the spring.

The crops were wilted to a DM concentration of approximately 300 g/kg and ensiled in bunker silos using a commercial acid-based additive (propionic and formic acids; ProMyrTM XR 630, Perstorp, Sweden) provided at a rate of 3.5 L/t. The experimental diets were formulated using the Lypsikki ration formulation system (Huhtanen and Nousiainen, 2014) to meet the ME and MP requirements for production of 35 kg of

ECM/d within the constraints of intake potential. A mixture of LS and barley was gradually replaced with ES (0, 33, 67, and 100% of the forage component of the diet) to obtain 4 diets defined as follows: late-cut (**L**), late-early (**LE**), early-late (**EL**), and early-cut (**E**) silage. With an increasing proportion of ES in the diet, the proportion of barley decreased from 47.2 to 26.6% on a DM basis. The changes in the proportion of total forage, barley, and 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, AarhusKarlshamn Ltd., Malmö, Sweden) was used as a protein supplement. Rolled barley was purchased from a local feed company (Fodercentralen, Umeå, Sweden). The formulation of the experimental diets is shown in Table 1.

Data and Sample Collections

Feed intake was recorded individually on a daily basis throughout the trial in roughage intake control feeders (Insentec B. V., Marknesse, the Netherlands) using 3 feeders for 4 cows. Only the data collected from d 15 to 28 of each period were used for the statistical analysis. The BW of the cows was recorded at the beginning of the study and subsequently after morning milking on d 24 to 26 in every experimental period. The BCS for each cow was measured by 2 people independently according to Edmonson et al. (1989) before the start of the study and at the end of each period.

Silage samples were collected twice per week and concentrate samples were collected once per week and pooled per period. All feed samples were oven dried at 60°C, and silage samples were frozen at -20°C. The diets were adjusted twice weekly to account for the changes in DM content. The dried samples were milled to pass through a 2- or 1-mm sieve for analytical purposes. The frozen samples were ground in a cutter mill (SM 2000, Retsch Ltd., Haan, Germany) to pass through a 20-mm sieve and stored in the freezer before analysis of silage fermentation parameters.

Apparent diet digestibility was assessed by collecting fecal grab samples (300 mL) from the rectum of 8 cows (that represented 2 squares) at 0600 and 1500 h on d 24 to 26 and kept at 4°C. Composite fecal samples per cow and period were obtained at the end of each collection period and oven dried at 60°C for 48 h. The samples were milled using a cutter mill to pass through an either 1-mm (chemical analysis) or 2-mm (digestibility marker) sieve for further analysis. Ash-free indigestible NDF (**iNDF**) concentration was used as an internal marker to calculate diet digestibility (Huhtanen et al., 1994), and potential digestible NDF (**pdNDF**) was calculated as $\text{NDF} - \text{iNDF}$.

Table 1. Ingredient composition of the formulated diets¹ (g/kg of DM)

Item	Diet ²			
	L	LE	EL	E
Late-cut silage	421	329	189	0
Early-cut silage	0	164	378	641
Rolled barley	472	404	335	266
Heat-treated canola meal expeller	97.0	93.0	89.0	84.0
NaCl	3.2	3.2	3.1	3.1
CaCO ₃	6.6	6.2	5.8	5.3

¹In addition to a TMR, the cows received a concentrate mixture during the visits to the GreenFeed system (C-Lock Inc., Rapid City, SD).

²A 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.

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 in every period at morning and evening milkings on 4 consecutive days from evening milking on d 19 until morning milking on d 21 and similarly on d 26 to 28. Gas emissions (CH₄ and CO₂) were measured using a transportable open-circuit head chamber system (GreenFeed system, C-Lock Inc., Rapid City, SD) as described by Huhtanen et al. (2015) and Hammond et al. (2016). Span gas calibrations (N₂ and a mixture of CH₄ and CO₂) were performed once a week, and CO₂ recovery tests were conducted every second week during each experimental period. Average recovery was $101 \pm 1.1\%$ (SE). Air flow was maintained above 26 L/s by cleaning the filters when the flow rate started to approach this level (alarm from C-Lock Inc.). The GreenFeed system was programmed to allow each animal to visit the 2 units at 5-h (minimum) intervals. Animals were given 8 servings of 50 g of commercial concentrate (Solid 220, Lantmännen, Malmö, Sweden) at 40-s intervals during each visit. The 2 GreenFeed units were operated continuously during the experimental period, but gas data are reported only for the last 14 d of each period. Average concentrate intake from GreenFeed was 1.4 kg of DM/d.

Chemical Analysis

Both feed and fecal samples were analyzed for DM (105°C for 16 h) and ash concentration by incinerating at 500°C for 4 h (AOAC International, 2012). 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). Ether extract in concentrate feeds was assessed according to method B in the Official Journal of the European

Communities (1984). 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 digestion unit (Ankom Technology Corp., Macedon, NY).

Indigestible NDF concentration in DM (Huhtanen et al., 1994) was determined by in situ incubation for 288 h according to Krizsan et al. (2015) and expressed as ash-free iNDF. The incubations were performed in 3 rumen-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). Starch intake was calculated using tabulated values for concentrate feeds (LUKE, 2016) and assuming that grass silage contained no starch.

The frozen silage samples were thawed and pressed, and the pH was measured with a pH meter (Metrohm, Herisa, Switzerland). Ammonium N was analyzed by direct distillation after adding MgO using a Kjeltac 2100 Distillation Unit (Foss). In addition, VFA and lactic acid were analyzed according to Ericson and André (2010). Concentrations of fat, CP, urea, and lactose in milk were measured by infrared spectroscopy (MilkoScan TM FT120, Foss), and the analytical values for each individual milking were weighted according to yield (to get the daily value for milk composition, a sum by product was performed in order to adjust composition, taking into account the milk yield recorded in the morning and in the afternoon milking) for a representative sample.

Calculations

Chemical composition and feeding values of the diets were calculated from the proportion of ingredients and their respective values. Total-tract apparent digestibility of DM and OM was calculated using iNDF as an internal marker in feeds and feces (Huhtanen et al., 1994). Fecal output of fermentable OM (kg/kg of DMI) was also calculated based on intake and digestibility values (fecal OM output – iNDF intake). Metabolizable energy concentration of the silages was calculated assuming 16 MJ of ME/kg of digestible DOM (MAFF, 1975). The concentration of DOM in silage was estimated from the iNDF and NDF concentrations using the equation of Huhtanen et al. (2013). For the concentrate ingredients, ME concentration was calculated from analyzed composition and tabulated digestibility coefficients. Concentrations of MP and rumen protein balance value (PBV) were calculated from analyzed composition using the coefficients in the Finnish feed tables (LUKE, 2016). Neutral detergent solubles (NDS) were calculated as OM – NDF. Energy-corrected milk

was calculated according to Sjaunja et al. (1990). Feed efficiency was calculated as ECM yield (kg/d)/DMI (kg/d), and milk N efficiency (MNE) was calculated as milk N (CP yield/6.38; g/d)/N intake (kg/d). Methane and CO₂ production was calculated as mean daily production during the last 14 d of each period.

Statistical Analysis

The experimental data were analyzed by ANOVA for a replicated 4 × 4 Latin square design using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). All data were pooled per cow and period. The statistical model was

$$Y_{ijkl} = \mu + S_i + P_j + C_k(S_i) + T_l + E_{ijkl},$$

where Y_{ijkl} is a dependent variable, μ is the mean for all observations, S_i is the effect of square i , P_j is the effect of period j , $C_k(S_i)$ is the effect of cow k within square i , T_l is the effect of diet l , and $E_{ijkl} \sim N(0, \sigma_e^2)$ represents the residual error. Interactions $P_j \times S_i$ and $P_j \times T_l$ were excluded from the final model because they were nonsignificant ($P \geq 0.10$). Apparent diet digestibility was analyzed by the following statistical model:

$$Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk},$$

where Y_{ijk} is a dependent variable, μ is the mean for all observations, P_i is the effect of period i , C_j is the effect of cow j , T_k is the effect of diet k , and $E_{ijk} \sim N(0, \sigma_e^2)$ represents the residual error.

To compare the effects of the graded addition of ES replacing LS and barley in the diet, linear and quadratic contrasts were used. Differences were declared statistically significant at $P \leq 0.05$.

RESULTS

Experimental Feeds and Diets

The concentration of CP decreased by 40 g/kg of silage DM and NDF concentration increased by 30 g/kg of silage DM in response to the later forage harvesting time (Table 2). The silage iNDF concentration also increased with advancing forage maturity. Silage fermentation quality during the experimental trial was good for both ES and LS silages as indicated by the low pH (mean = 3.78) and low concentrations of VFA and ammonia N.

The main difference in diet composition between treatments was the replacement of starch from barley with forage pdNDF by increasing the proportion of ES

Table 2. Chemical composition of the experimental dietary ingredients (g/kg of DM unless otherwise stated)

Item	Silage ¹		Concentrate feed		
	ES	LS	Rolled barley	Canola meal	Concentrate mix ²
DM, g/kg	293	327	902	907	880
In DM, g/kg					
Ash	70.0	56.9	25.4	72.6	51.6
CP	179	139	122	390	202
Ether extract	37.0	32.0	24.1	87.0	60.0
NDF	575	605	188	367	197
Indigestible NDF	80	137	43	128	77
Starch	—	—	503	16	357
NFC	139	167	641	83	489
Neutral detergent solubles	355	338	787	560	751
Fermentation quality					
pH	3.89	3.67			
In DM, g/kg					
Lactic acid	99.6	86.1			
Acetic acid	21.6	12.2			
Propionic acid	1.1	1.5			
Butyric acid	0.27	0.23			
Formic acid	5.01	6.72			
Ethanol	7.41	13.2			
2,3-Butanediol	0.5	1.0			
Ammonium N, g/kg of N	40.5	23.1			
Feeding values					
ME, ³ MJ/kg of DM	11.0	9.7	13.2	12.3	12.9
MP, g/kg of DM	85.7	73.9	97.8	171	120
PBV, ⁴ g/kg of DM	51.7	29.1	−20.2	117	21.0

¹LS = late-cut silage; ES = early-cut silage.²Concentrate mixture used in GreenFeed (Solid 220, Lantmännen, Malmö, Sweden).³Based on coefficients from feed tables (LUKE, 2016).⁴PBV = protein balance in the rumen.

in the diet (Table 3). Because of the differences in silage CP concentration between LS and ES, the dietary CP concentration increased with the proportion of ES in the diet. Differences between the diets in calculated ME and MP concentrations were small, but calculated PBV increased with the inclusion of ES.

Nutrient Intake

The proportion of concentrate on a DM basis, including the commercial mixture (Solid 220) provided by the GreenFeed system, was 0.62, 0.57, 0.50, and 0.46 for the L, LE, EL, and E diets, respectively (Table 4). Differences in nutrient intakes among treatments were closely associated with linear rather than quadratic changes in intake responses. Total DM, OM, and starch intake decreased linearly ($P < 0.01$) with increasing proportion of ES. Diet had no effect ($P \geq 0.16$) on CP and NDF intake, but the intake of iNDF decreased in response to increasing inclusion of ES. The quadratic effect of starch intake was significant ($P = 0.03$); however, the linear trend explained 98.6% of the variation in starch intake. Intake of MP decreased linearly ($P < 0.01$),

Table 3. Chemical composition and calculated feeding values of the experimental diets (g/kg of DM unless otherwise stated)

Item ¹	Diet ²			
	L	LE	EL	E
In DM, g/kg				
OM	947	944	940	936
CP	157	164	171	180
Ether extract	34.8	36.3	37.7	39.3
NDF	364	382	401	416
iNDF	93	90	85	78
pdNDF	271	292	316	338
NDS	583	562	539	520
NFC	380	353	324	298
Starch	250	222	194	168
Feeding values				
ME, MJ/kg of DM	11.6	11.6	11.7	11.8
MP, g/kg of DM	95.6	96.1	96.7	97.9
PBV, ³ g/kg of DM	14.4	20.3	26.8	34.0

¹iNDF = indigestible NDF; pdNDF = potentially digestible NDF; NDS = neutral detergent solubles (OM − NDF).²Proportions of late-cut and early-cut silage in the forage component of the diet: L = late-cut silage (100:0); LE = late-early silage (67:33); EL = early-late silage (67:33); E = early-cut silage (0:100). Includes concentrate intake from the GreenFeed system (C-Lock Inc., Rapid City, SD).³PBV = protein balance in the rumen.

Table 4. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage on nutrient intakes (kg/d unless otherwise stated)

Intake ¹	Diet ²				SEM	Contrast ³	
	L	LE	EL	E		Lin	Quad
Total DM	22.6	20.8	20.2	19.3	0.51	<0.01	0.16
Concentrate DM	14.0	11.8	10.2	8.9	0.25	<0.01	0.05
OM	21.5	19.6	19.0	18.1	0.48	<0.01	0.16
CP	3.55	3.40	3.45	3.48	0.09	0.58	0.16
Ether extract	0.79	0.75	0.76	0.76	0.02	0.19	0.25
NDF	8.25	7.96	8.11	8.03	0.23	0.40	0.42
iNDF	2.10	1.89	1.72	1.50	0.05	<0.01	0.90
pdNDF	6.15	6.09	6.38	6.54	0.18	<0.01	0.34
NDS	13.2	11.6	10.9	10.0	0.26	<0.01	0.08
Starch	5.66	4.60	3.92	3.24	0.10	<0.01	0.03
ME, ⁴ MJ/d	263	242	236	227	5.73	<0.01	0.10
ME _{DOM} , MJ/d	225	212	210	207	5.36	<0.01	0.15
MP	2.16	1.99	1.95	1.89	0.05	<0.01	0.12
PBV	0.33	0.42	0.54	0.66	0.02	<0.01	0.52

¹iNDF = indigestible NDF; pdNDF = potentially digestible NDF; NDS = neutral detergent solubles; ME_{DOM} = ME calculated from digestible OM (16 MJ/kg); PBV = protein balance in the rumen.

²Proportions of late-cut and early-cut silage in the forage component of the diet: L = late-cut silage (100:0); LE = late-early silage (67:33); EL = early-late silage (67:33); E = early-cut silage (0:100). Includes concentrate intake from the GreenFeed system (C-Lock Inc., Rapid City, SD).

³Lin = linear effect of dietary addition of early-cut silage; Quad = quadratic effect of dietary addition of early-cut silage.

⁴Based on coefficients from feed tables (LUKE, 2016).

and intake of PBV increased with increasing proportion of ES in the diet. Intake of ME at maintenance level and the intake of ME estimated based on DOM both decreased linearly ($P < 0.01$) as the proportion of ES increased in the diet, but the decline was greater when estimated from determined DOM intake.

Digestibility and Fecal Output

The effects of increased inclusion of ES in the diet on diet digestibility and fecal output are shown in Table 5. Overall, digestibility of nutrients increased linearly ($P < 0.01$) with increasing inclusion of high-quality

Table 5. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage on apparent digestibility of the diet and fecal output

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
Digestibility, g/kg							
DM	645	664	680	705	6.7	<0.01	0.60
OM	656	675	691	715	6.7	<0.01	0.69
CP	604	625	676	704	10.1	<0.01	0.75
NDF	497	537	565	621	11.4	<0.01	0.50
pdNDF ³	665	703	719	759	14.4	<0.01	0.77
NDS ⁴	755	769	782	788	6.4	<0.01	0.51
Fecal output, g/kg of DMI							
CP	62.4	61.4	55.5	53.4	1.69	<0.01	0.73
NDS	143	131	118	111	3.70	<0.01	0.42
pdNDF	90.2	85.9	87.1	78.0	3.77	0.05	0.53
NDS-CP	80.9	69.1	62.7	57.7	2.35	<0.01	0.12

¹Proportions of late-cut and early-cut silage in the forage component of the diet: L = late-cut silage (100:0); LE = late-early silage (67:33); EL = early-late silage (67:33); E = early-cut silage (0:100).

²Lin = linear effect of dietary addition of early-cut silage; Quad = quadratic effect of dietary addition of early-cut silage.

³pdNDF = potentially digestible NDF (NDF – indigestible NDF).

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

grass silage in the diet. The greatest numerical differences in digestibility between the L and E diets were observed for NDF and CP, which accounted for 124 and 100 g/kg, respectively. The digestibility of NDS was greater in diets containing ES. Fecal output of nutrients decreased linearly ($P < 0.01$) as a result of improvements in forage quality supply by graded addition of ES to the diet.

Milk Production and Feed Efficiency

Milk yield was not influenced by diet (Table 6). Milk fat concentration increased and protein concentration decreased linearly ($P = 0.05$) with increasing proportion of ES in the diet. Milk protein yield decreased ($P < 0.05$) with increased inclusion of ES. The concentration of MUN increased linearly ($P < 0.01$) as the ES level increased in the diet. Milk fat yield was not influenced by the diet, but milk protein yield decreased linearly ($P = 0.05$) as the proportion of ES increased. Feed efficiency, expressed as ECM/DMI, increased linearly ($P < 0.01$) with increasing ES in the diet, but MNE was similar among treatments.

Gas Emissions

Two cows during 2 periods did not visit the GreenFeed system, leaving 60 observations for statistical analysis. On average, the cows visited the GreenFeed system 56 times during the 14-d observation period. The average ($\pm$ SD) duration of visits was 4.05 (± 1.05), 4.07 (± 1.08), 3.93 (± 1.10), and 3.95 (± 0.98) min for diets L, LE, EL, and E, respectively. Duration of visit was not related to observed CH_4 emission during the visit ($R^2 = 0.0012$). Diurnal patterns in CH_4 emissions were consistent among the treatments, and the pattern of changes was similar among diets (Figure 1). Total CH_4 emissions averaged 443 g/d and did not differ between the experimental diets (Table 7). Methane yield increased in response to the addition of ES to the diet, but CH_4 intensity (g of CH_4 /kg of ECM) was not influenced by the diet. Diet had no significant effect ($P > 0.10$) on total CO_2 emissions or CH_4 : CO_2 ratio.

DISCUSSION

The effects of gradually increasing the proportion of ES to replace LS and barley were studied to evaluate

Table 6. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage on milk production and feed efficiency

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
Yield							
Milk, kg/d	28.5	28.1	27.9	27.7	0.72	0.16	0.80
ECM, ³ kg/d	30.0	29.7	29.7	29.7	0.79	0.55	0.72
Fat, g/d	1,216	1,209	1,221	1,229	35.6	0.60	0.74
Protein, g/d	1,047	1,022	1,001	996	26.2	<0.01	0.46
Lactose, g/d	1,318	1,312	1,305	1,292	38.5	0.32	0.83
Concentration							
Fat, g/kg	43.0	43.4	43.9	44.7	0.88	0.05	0.73
Protein, g/kg	37.0	36.7	36.2	36.3	0.57	0.05	0.43
Lactose, g/kg	46.4	46.8	46.9	46.8	0.60	0.15	0.17
MUN, mg/dL	9.7	10.1	11.1	11.9	0.56	<0.01	0.34
Feed efficiency							
ECM/DMI, kg/kg	1.33	1.44	1.48	1.54	0.05	<0.01	0.40
MNE, ⁴ g/kg	296	302	291	288	9.83	0.13	0.36
N excess, ⁵ g/kg of ECM	13.6	13.0	13.5	13.7	0.67	0.61	0.20
FFOM, ⁶ g/kg of ECM	170	148	135	120	8.20	<0.01	0.56
BW, kg	631	633	632	629	17.0	0.74	0.51
BCS, units	3.14	3.16	3.14	3.19	0.11	0.48	0.76

¹Proportions of late-cut and early-cut silage in the forage component of the diet: L = late-cut silage (100:0); LE = late-early silage (67:33); EL = early-late silage (67:33); E = early-cut silage (0:100).

²Lin = linear effect of dietary addition of early-cut silage; Quad = quadratic effect of dietary addition of early-cut silage.

³Calculated according to Sjaunja et al. (1990).

⁴MNE = milk N/N intake.

⁵N excess = [N intake (g/d) – milk N yield (g/d)]/ECM yield (kg/d).

⁶FFOM = [(potentially digestible NDF fecal output (g/kg of DMI) + neutral detergent solubles output (g/kg of DMI)) $\times$ DMI (kg/d)]/ECM (kg/d) $\times$ 1,000.

Figure 1. Diurnal pattern of CH₄ emissions in cows offered 4 diets. Proportions of late-cut and early-cut silage in the forage component of the diet were as follows: L = 100:0; LE = 67:33; EL = 67:33; E = 0:100. Standard deviation for all visits at different time points ranged from 8.5 g/h (at 2100 h) to 10.4 g/h (at 1700 h).

whether improvements in forage quality can reduce concentrate supplementation without compromising animal performance and N efficiency or increasing CH₄ emissions. The 4 diets were formulated to support the same ECM yield. The purpose of this study was not

to compare production responses to the level of concentrate supplementation or forage quality as isolated factors but rather to examine the effects of replacing a mixture of late-harvested silage and barley with early-harvested silage on performance and environmental

Table 7. Effects of graded replacement of late-cut silage and rolled barley with early-cut silage on methane (CH₄) and carbon dioxide (CO₂) emissions

Item	Diet ¹				SEM	Contrast ²	
	L	LE	EL	E		Lin	Quad
Visits to GF ³	49	54	59	63	8.0	0.03	0.91
Concentrate DMI, ⁴ kg/d	1.2	1.3	1.4	1.5	0.21	0.03	0.91
CH ₄ g/d	440	441	446	444	13.2	0.71	0.94
g/kg of DMI	19.5	21.3	22.1	23.0	0.69	<0.01	0.28
g/kg of ECM	14.8	15.0	15.2	15.2	0.63	0.46	0.92
CO ₂ g/d	13,271	13,012	13,152	12,949	302	0.23	0.85
g/kg of DMI	588	631	656	670	17.0	<0.01	0.10
g/kg of ECM	448	443	450	444	13.4	0.84	0.95
CH ₄ /CO ₂ , g/kg	33.2	33.6	33.8	34.2	0.57	0.17	0.97

¹Proportions of late-cut and early-cut silage in the forage component of the diet: L = late-cut silage (100:0); LE = late-early silage (67:33); EL = early-late silage (67:33); E = early-cut silage (0:100).

²Lin = linear effect of dietary addition of early-cut silage; Quad = quadratic effect of dietary addition of early-cut silage.

³Total number of visits to the GreenFeed system (C-Lock Inc., Rapid City, SD) during the 14-d collection period.

⁴Concentrate DMI from the GreenFeed system.

impact. Production responses to forage quality and level of concentrate supplementation in dairy cows fed grass silage-based diets have been studied previously using changeover designs with factorial arrangements (Aston et al., 1994; Kuoppala et al., 2008; Randby et al., 2012).

Diet formulation was successful, as the differences in ECM yield between the treatments were minor. The differences between ES and LS in dietary concentrations of DOM and ME were as expected, but they were lower than expected in both silages, probably because of exceptionally warm weather conditions during early summer. The decline in predicted *in vivo* DOM concentration (5.6 g/d) with advancing maturity agrees with the corresponding values of 5.0 and 6.2 g/d for primary growth grass silages (Kuoppala et al., 2008; Randby et al., 2012). Differences in DM concentration and fermentation quality were small between the silages. These differences were judged not to be of relevance with regard to forage and total DMI potential (Huhtanen et al., 2007).

Feed Intake and Diet Digestibility

It is well known that concentrate supplementation decreases silage DMI and, as a consequence, increases total DMI. In the present study, the difference in DMI between L and E diets (3.3 kg/d) was greater than expected (Table 4) but was consistent with observed intakes in a parallel physiological study we conducted in tie stalls with manual TMR feeding of the same diets (E. H. Cabezas-Garcia, S. J. Krizsan, K. J. Shingfield, and P. Huhtanen, unpublished data). The model developed by Huhtanen et al. (2008) predicted a smaller difference of 1.7 kg/d in DMI between the L and E diets, but the predicted intake potential of the diets was strongly correlated with observed DMI ($R^2 = 0.98$). The substitution rate (decline in forage intake per unit of DMI) was 0.37, which is within the range reported by Thomas (1987), Rook et al. (1991), and Huhtanen et al. (2008) on analysis of data from studies covering most practical on-farm feeding situations across Europe when the concentrate supplementation was increased with the same silage. In the present study, whole barley kernels were observed in feces, indicating inadequate grain processing of barley, especially when the proportion of LS and barley in the diet increased. This observation was consistent with greater fecal outputs of NDS and NDS-CP fraction per kilogram of DMI and could have enhanced the depression in diet digestibility more than expected from changes in diet composition. Reduced energy from concentrate implies that the cows increased DMI to meet the energy requirement. In agreement with this hypothesis, reduced starch digest-

ibility of grain-based concentrate increased silage DMI in a study by Jaakkola et al. (2009).

Rumen evacuation data in our companion study with the same diets indicated that rumen fill was not a limiting factor for DMI in the diets based on increasing proportion of ES, as suggested by the reduced rumen NDF pool size with increased inclusion of ES (E. H. Cabezas-Garcia, S. J. Krizsan, K. J. Shingfield, and P. Huhtanen, unpublished data). Rinne et al. (2002) observed increased DMI of early-cut silage accompanied by a decreased ruminal fiber pool when cows were fed grass silages differing in maturity and supplemented with a fixed level of concentrate. In other studies, rumen fill also decreased with improved silage digestibility (e.g., Gasa et al., 1991; Bosch et al., 1992).

Reduced apparent diet digestibility with increased proportion of LS and barley was greater than predicted from forage DOM concentration and tabulated coefficients for concentrates (LUKE, 2016). As a result, the differences between the diets in calculated ME intake were greater than when estimated from observed DOM intake (36 and 18 MJ of ME/d for the LS and ES diets, respectively; Table 4). This difference can be attributed partly to negative associative effects with increased concentrate supplementation and partly to increased fecal NDS output due to poor grain processing.

In the present study, the difference between the L and E diets in fecal output of NDS was 33 g/kg of DMI, whereas in the meta-analysis by Nousiainen et al. (2009) DMI or diet composition did not affect fecal output of NDS. Therefore, the observed difference was most likely attributable to excretion of whole barley kernels in feces. Part of the reduction in diet digestibility with increasing proportion of LS and barley can be related to increased passage rate with higher DMI. With increased feeding level, the depression in digestibility is greater for diets containing alfalfa hay and cracked corn at high concentrate levels than for those containing alfalfa hay and cracked corn at low concentrate levels (Colucci et al., 1989). In Colucci et al. (1989), in addition to reduced digestibility of NDF, the starch contributed to depressing diet digestibility at high DMI. Energy supplements are usually more digestible than forages, and thus increased concentrate supplementation could be expected to improve total diet digestibility. However, increased concentrate supplementation in dairy cows fed high-quality forages had no influence on diet digestibility in previous single studies (e.g., Aston et al., 1994; Randby et al., 2012; Kuoppala et al., 2008) or in a meta-analysis (Nousiainen et al., 2009).

Postponing the grass harvesting time increased iNDF concentrations in the silage from 80 to 137 g/kg of DM (Table 2), which was reflected in increased

iNDF concentration in the whole diet. In the present study, lower digestibility of pdNDF with increasing LS and barley in the diet can be related partly to intrinsic forage characteristics (digestion rate of pdNDF) and partly to negative associative effects on cell wall digestion. Digestion rate of pdNDF derived from evacuation data decreased with increasing proportion of LS and barley in the diet (E. H. Cabezas-Garcia, S. J. Krizsan, K. J. Shingfield, and P. Huhtanen, unpublished data). In line with this observation, digestion rate of pdNDF has been found to decrease with increased concentrate proportion in rumen evacuation studies (Huhtanen and Jaakkola, 1993; Stensig and Robinson, 1997).

Milk Production, Milk Composition, and Feed Efficiency

Dietary treatments had no effect on milk yield or ECM yield. Because the effects of silage digestibility and proportion of concentrate were confounded due to the experimental objectives, production responses to these factors cannot be compared directly with values from the literature. In the present study, an increase of 10 g/kg of DM in silage DOM concentration had the same productive value as 0.64 kg of concentrate DM. This value compares well with that (0.81) estimated by Huhtanen et al. (2013) from factorial studies evaluating the effects of silage digestibility and concentrate supplementation. In the study by Ferris et al. (2001), milk yield increased with enhanced concentrate level with both medium- and high-quality silages, but on average 3 kg/d less concentrate DM was required to reach the same milk yield with medium-quality compared with high-quality silage. Similar trends were observed by Kuoppala et al. (2008). Milk and especially ECM yield responses diminish with increased concentrate supplementation and in some cases can be negative (Ferris et al., 2001; Randby et al., 2012).

Positive effects of increased concentrate intake on milk protein concentration and yield were most likely related to increased supply of ME. The increase in milk protein yield per incremental DMI was 15 g/kg. This value is low compared with, for example, Kuoppala et al. (2008). In their study the corresponding response was 50 g/kg for 2×2 factorial combination of early- and late-harvested primary growth grass silage and 2 levels of concentrate, indicating poor utilization of incremental intake in the current study.

Milk fat concentration linearly increased with graded inclusion of ES in the diet in the present study. The difference between L and E diets in fat concentration was 1.7 g/kg, which agrees with the study by Kuoppala et al. (2008). In their study, milk fat concentration was 4

g/kg higher in cows fed early-cut silage with a low level of concentrate than in cows fed late-cut silage with a high level of concentrate. In the study by Randby et al. (2012), there were linear trends toward decreases in milk fat concentration with postponed silage harvest time and increased concentrate intake, but the interaction of these 2 factors was not significant. Overall, the reported effects of forage digestibility and level of concentrate supplementation on milk fat concentration have not been consistent and tend to be more related to forage quality than concentrate supplementation. Only concentrate levels of 70% on a DM basis consistently decreased milk fat content regardless of forage quality (Ferris et al., 2001). The effects of concentrate level on milk fat concentration in cows fed grass silage-based diets are small probably because increased starch intake has little influence on VFA pattern (Sveinbjörnsson et al., 2006).

The MUN concentration increased with the proportion of ES in the diet, reflecting increased dietary CP concentration. The CP concentration in the diet is the most important single factor affecting MUN concentration (Broderick and Clayton, 1997; Nousiainen et al., 2004). The effect of dietary CP concentration on MUN was smaller than that reported by Nousiainen et al. (2004) for data derived from 50 milk production trials (0.10 compared with 0.17 mg/g of CP), probably because the increase in dietary CP concentration was associated with higher digestibility and ME concentration.

Feed efficiency (ECM/DMI) increased linearly with increasing replacement of LS and barley (concentrate and less-digestible fiber) with ES (more digestible fiber; Table 6). This could indicate that the increase in ME supply was less than calculated because of unexpected loss of grain in feces. Another possible explanation for reduced apparent feed efficiency with increased LS and barley is increased partitioning of nutrients to body tissues. A trend for increased milk protein concentration suggests that the cows fed increasing amounts of barley had a more positive energy balance. However, no differences were detected in either BW or BCS.

Nitrogen utilization efficiency did not differ significantly between the diets despite increased dietary CP concentration with increased proportion of ES in the diet. The models of Huhtanen and Hristov (2009) based on dietary CP concentration and milk yield predicted a 30 g/kg decrease in MNE with increased proportion of ES in the diet. However, their model based on the concentrations of CP and TDN predicted similar values of MNE for all diets (276–279 g/kg). This indicates that the effects of increased CP concentration with ES was compensated for by improved diet digestibility.

The difference between L and E diet in dietary CP concentration was 14.2% but only 6.4% in a CP:OM digestibility ratio. Similar N excess per kilogram of ECM is consistent with Kebreab et al. (2001), who reported that fecal and urinary N losses were closely related to N intake. The negative relationship between MUN and MNE was consistent with that reported in the meta-analysis by Nousiainen et al. (2004). The study by Castillo et al. (2001) also found a strong negative relationship between N intake and MNE. Keady and Mayne (1998) found that increased level of concentrate in the diet provided more readily fermentable energy in the rumen, which improved the utilization of degradable N from the silage and other dietary components and subsequently increased milk protein yield.

Gas Emissions

Total CH₄ emissions and CH₄ intensity did not differ between the diets, but CH₄ yield increased with the proportion of ES in the diet. On a GE basis (18.5 MJ of GE/kg of DM), total CH₄ emissions ranged from 57.9 for the L diet to 68.3 kJ/MJ for the E diet. These values are within the range reported for grass silage-based diets for dairy cows (Yan et al., 2000). The greater CH₄ yield with increased proportion of ES in the diet can partly be attributed to lower DMI and greater OMD compared with LS diets. Observed values of CH₄ yield were closely correlated with the values predicted by the model (equation 17) of Ramin and Huhtanen (2013), but the range in predicted values, assuming that OMD at maintenance level was 40 g/kg higher than observed OMD, was narrower (from 18.9 to 20.8 g of CH₄/kg of DMI).

Concentrate can be expected to reduce CH₄ emissions in cattle because fermentation of starch produces less CH₄ than NDF in rumen conditions. With high-concentrate (>90% of DMI) feedlot-type diets, CH₄ yield is only 2 to 3% of GE intake (Johnson and Johnson, 1995). However, within the range of concentrate supplementation in grass silage-based diets for dairy cows (37–70% of DM), the differences between the diets in CH₄ energy losses are marginal until 59% and tend to decrease only at the highest levels (~70%) of supplementation (Ferris et al., 2001). In line with this observation, the meta-analysis by Ramin and Huhtanen (2013), based on 298 treatment means, suggests that the effect of dietary carbohydrate composition on CH₄ yield is small for diets with <75% of concentrates on a DM basis. An increased proportion of concentrate has occasionally been reported to increase CH₄ yield. For example, in the study by Beever et al. (1988), gradual replacement of late-cut grass silage with barley at a rate from 0 to 560 g/kg of DM in the diet of growing

beef cattle increased CH₄ yield from 6.7 to 8.2% of GE intake.

The models proposed by Blaxter and Clapperton (1965) indicate that improved diet digestibility is positively related to CH₄ production at maintenance level but that the response diminishes with increased feeding level. The more recent meta-analysis study by Ramin and Huhtanen (2013) shows a positive relationship between digestibility and CH₄ yield. However, the effects of silage digestibility on CH₄ yield are inconsistent. In the study by Beever et al. (1988), CH₄ yield was greater (7.3 compared with 6.7% of GE intake) with early-cut, high-digestibility silage than with late-cut, low-digestibility silage. In contrast, Gordon et al. (1995), Brask et al. (2013), and Warner et al. (2016) reported a lower CH₄ yield in dairy cows fed diets containing early-cut ryegrass silages than in those fed diets containing late-cut ryegrass silages. In zero grazing, digestibility of grass had no effect on CH₄ yield in beef heifers (Hart et al., 2009) or dairy cows (Warner et al., 2015).

In addition to mitigation strategies focusing on reducing enteric CH₄ emissions, gas production from manure should be also considered. Methane emissions from manure were not measured in the present study, but greater fecal concentrations (44 g/kg of DMI) and output (1.4 kg/d) of potentially digestible nutrients (NDS + pdNDF) with increasing proportion of LS and barley in the diet could counterbalance reduced enteric CH₄ yield. Møller et al. (2014) found a negative relationship between enteric CH₄ yield and fecal CH₄ potential. In dairy goats, the potential CH₄ production from incubated feces was much greater (5.9 compared with 0.3 L of CH₄/kg of OM) for high-starch diets compared with low-starch diets (Ibáñez et al., 2015). These observations are in line with the results obtained in the present study and suggest that fecal CH₄ potential is related to biodegradability of OM in manure.

CONCLUSIONS

The results of this study indicate that it is possible to replace late-cut silage and rolled barley with early-cut silage without compromising animal performance and N efficiency or increasing CH₄ emissions. Milk protein yield was reduced, but feed efficiency was improved with increased proportion of ES in the silage. Overall, dairy cows fed diets with an increased proportion of early-cut grass silage had lower DMI, but it was counterbalanced by higher digestibility of the nutrients, which in turn reduced the need for concentrate supplementation. Increased proportion of concentrates with LS can increase diet cost without improvements in CH₄ intensity or N efficiency.

ACKNOWLEDGMENTS

The authors express their appreciation to the Röbäcksdalen farm crew (Swedish University of Agricultural Sciences, Umeå, Sweden) for making silage and caring for the dairy cows. Thanks to Ann-Sofi Hahlin and Lars Wallgren at the Department of Agricultural Research for Northern Sweden (Umeå, Sweden) for their laboratory assistance. This work was supported by RuminOmics (project no. 289319 of the European Community 7th Framework Programme: Food, Agriculture, Fisheries and Biotechnology).

REFERENCES

- AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International, Gaithersburg, MD.
- Aston, K., C. Thomas, S. R. Daley, J. D. Sutton, and M. S. Dhanoa. 1994. Milk production from grass silage diets: Effects of silage characteristics and the amount of supplementary concentrate. *Anim. Prod.* 59:31–41.
- Beever, D. E., S. B. Cammell, C. Thomas, M. C. Spooner, M. J. Haines, and L. Gale. 1988. The effect of date of cut and barley substitution on gain and on the efficiency of utilization of grass silage by growing cattle. *Br. J. Nutr.* 60:307–319.
- Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. *Br. J. Nutr.* 19:511–522.
- Bosch, M. W., S. C. W. Lammers-Wienhoven, G. A. Bangma, H. Boer, and P. W. M. van Adrichem. 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particles and mastication activity. *Livest. Prod. Sci.* 32:265–281.
- Brask, M., P. Lund, A. L. F. Hellwing, M. Poulsen, and M. R. Weisbjerg. 2013. Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. *Anim. Feed Sci. Technol.* 184:67–79.
- Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964–2971.
- Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France. 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* 79:240–246.
- Colucci, P. E., G. K. Macleod, W. L. Grovum, L. W. Cahill, and I. McMillan. 1989. Comparative digestion in sheep and cattle fed different forage to concentrate ratios at high and low intakes. *J. Dairy Sci.* 72:1774–1785.
- Czerkawski, J. W. 1986. An Introduction to Rumen Studies. Robert Maxwell, Oxford, United Kingdom.
- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72:68–78.
- Ericson, B., and J. André. 2010. HPLC—Applications for agricultural and animal science. Pages 23–26 in *Proc. 1st Nordic Feed Sci. Conf.* Uppsala, Sweden. The Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Ferris, C. P., F. J. Gordon, D. C. Patterson, D. J. Kilpatrick, C. S. Mayne, and M. A. McCoy. 2001. The response of dairy cows of high genetic merit to increasing proportion of concentrate in the diet with a high and medium feed value silage. *J. Agric. Sci.* 136:319–329.
- Ferris, C. P., F. J. Gordon, D. C. Patterson, M. G. Porter, and T. Yan. 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilization by lactating dairy cows. *J. Agric. Sci.* 132:483–490.
- Gasa, J., K. Holtenius, J. D. Sutton, M. S. Dhanoa, and D. J. Nappers. 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage and lib. *Br. J. Nutr.* 66:381–398.
- Gordon, F. J., M. G. Porter, C. S. Mayne, E. F. Unsworth, and D. J. Kilpatrick. 1995. Effect of forage digestibility and type of concentrate on nutrient utilization by lactating dairy cattle. *J. Dairy Res.* 62:15–27.
- Hammond, K. J., G. C. Waghorn, and R. S. Hegarty. 2016. The Green-Feed system for measurement of enteric methane emission from cattle. *Anim. Prod. Sci.* 56:181–189.
- Hart, K. J., P. G. Martin, P. A. Foley, D. A. Kenny, and T. M. Boland. 2009. Effect of sward dry matter digestibility on methane production, ruminal fermentation, and microbial populations of zero-grazed beef cattle. *J. Anim. Sci.* 87:3342–3350.
- Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special topics—Mitigation of the methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *J. Anim. Sci.* 91:5045–5069.
- Huhtanen, P., E. H. Cabezas-Garcia, S. Utsumi, and S. Zimmerman. 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. *J. Dairy Sci.* 98:3394–3409.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232.
- Huhtanen, P., and S. Jaakkola. 1993. The effects of forage preservation method and proportion of concentrate on digestion of cell wall carbohydrates and rumen digesta pool size in cattle. *Grass Forage Sci.* 48:155–165.
- Huhtanen, P., S. Jaakkola, and J. Nousiainen. 2013. An overview of silage research in Finland: From ensiling innovation to advances in dairy cow feeding. *Agric. Food Sci.* 22:35–56.
- Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given 6 different diets. *Anim. Feed Sci. Technol.* 48:211–227.
- Huhtanen, P., and J. Nousiainen. 2014. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Pages 71–90 in *Recent Advances Animal Nutrition—2013*. P. C. Garnsworthy and J. Wiseman, ed. Context, Packington, United Kingdom.
- Huhtanen, P., M. Rinne, and J. Nousiainen. 2007. Evaluation of the factors affecting silage intake of dairy cows: A revision of the relative silage dry-matter intake index. *Animal* 1:758–770.
- Huhtanen, P., M. Rinne, and J. Nousiainen. 2008. Evaluation of concentrate factors affecting silage intake of dairy cows: A development of the relative total diet intake index. *Animal* 2:942–953.
- Ibáñez, C., V. J. Moya, H. Arriaga, D. M. López, P. Merino, and C. Fernández. 2015. Replacement of cereal with low starch fibrous by-products on nutrients utilization and methane emissions in dairy goats. *Open J. Anim. Sci.* 5:198–209.
- Jaakkola, S., E. Saarisalo, and T. Heikkilä. 2009. Formic acid treated whole crop barley and wheat silages in dairy cow diets: Effects of crop maturity, proportion in the diet, and level and type of concentrate supplementation. *Agric. Food Sci.* 18:234–256.
- Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.* 73:2483–2492.
- Keady, T. W. J., and C. S. Mayne. 1998. The effects of concentrate energy source on silage feeding behavior and energy utilization by lactating dairy cows offered grass silages with differing intake characteristics. *Anim. Sci.* 67:225–236.
- Kebreab, E., J. France, D. E. Beever, and A. R. Castillo. 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutr. Cycl. Agroecosyst.* 60:275–285.
- Krizsan, S. J., M. Rinne, L. Nyholm, and P. Huhtanen. 2015. New recommendations for the ruminal in situ determination of indigestible neutral detergent fibre. *Anim. Feed Sci. Tech.* 205:31–41.
- Kuoppala, K., M. Rinne, J. Nousiainen, and P. Huhtanen. 2008. The effect of cutting time of grass silage in primary growth and re-

- growth and the interactions between silage quality and concentrate level on milk production of dairy cows. *Livest. Sci.* 116:171–182.
- LUKE. 2016. Finnish feed tables. Accessed May 3, 2016. https://portal.mtt.fi/portal/page/portal/Rehutaulukot/feed_tables_english.
- Ministry of Agriculture, Fisheries and Food (MAFF). 1975. Energy allowance and feeding systems for ruminants. Tech. bull. 33. Her Majesty's Stationery Office, London, United Kingdom.
- Møller, H. B., V. Moset, M. Brask, M. R. Weisbjerg, and P. Lund. 2014. Feces composition and manure derived methane yield from dairy cows: Influence of diet with focus on fat supplement and roughage type. *Atmos. Environ.* 94:36–43.
- Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. *J. Dairy Sci.* 80:1005–1028.
- Nousiainen, J., M. Rinne, and P. Huhtanen. 2009. A meta-analysis of feed digestion in dairy cows: 1. The effects of forage and concentrate factors on total diet digestibility. *J. Dairy Sci.* 92:5019–5030.
- Nousiainen, J., K. J. Shingfield, and P. Huhtanen. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *J. Dairy Sci.* 87:386–398.
- Official Journal of the European Communities. 1984. Determination of crude oils and fat. Method B. Office for Official Publications of the European Communities, Luxembourg City, Luxembourg.
- Ramin, M., and P. Huhtanen. 2013. Development of equations for predicting methane emissions from ruminants. *J. Dairy Sci.* 96:2476–2493.
- Randby, A. T., M. R. Weisbjerg, P. Nørgaard, and B. Heringstad. 2012. Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. *J. Dairy Sci.* 95:304–317.
- Rinne, M. 2000. Influence of the timing of the harvest of primary grass growth on herbage quality and subsequent digestion and performance in the ruminant animal. PhD Thesis. University of Helsinki, Finland.
- Rinne, M., P. Huhtanen, and S. Jaakkola. 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. *J. Anim. Sci.* 80:1986–1998.
- Rook, A. J., M. Gill, R. D. Willink, and S. J. Lister. 1991. Prediction of voluntary intake of grass silage by lactating dairy cows offered concentrates at a flat rate. *Anim. Prod.* 52:407–420.
- Sauvant, D., and S. Giger-Reverdin. 2009. Modelling of digestive interactions and methane production in ruminants. *INRA Prod. Anim.* 22:375–384.
- Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in *Proc. 27th Session Int. Committee of Recording and Productivity of Milk Animals*. EEAP publication no. 50. P. Gallion and Y. Chabert, ed. Pudoc, Wageningen, the Netherlands.
- Stensig, T., and P. H. Robinson. 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. *J. Dairy Sci.* 80:1339–1352.
- Sveinbjörnsson, J., P. Huhtanen, and P. Udén. 2006. The Nordic dairy cow model, Karoline—Development of volatile fatty acid submodel. Pages 1–14 in *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches*. E. Kebreab, J. Dijkstra, A. Bannink, W. J. J. Gerrits, and J. France, ed. CAB International, Wallingford, United Kingdom.
- Thomas, C. 1987. Factors affecting the substitution rates in dairy cows on silage based rations. Pages 205–218 in *Recent Advances in Animal Nutrition*. W. Haresign and D. J. A. Cole, ed. Butterworths, London, United Kingdom.
- Van Soest, P. J. 1994. *Nutritional Ecology of the Ruminant*. Cornell Univ. Press, Ithaca, NY.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Warner, D., B. Hatew, S. C. Podesta, G. Klop, S. van Gastelen, H. van Laar, J. Dijkstra, and A. Bannink. 2016. Effects of nitrogen fertilisation rate and maturity of grass silage on methane emissions by lactating dairy cows. *Animal* 10:34–43.
- Warner, D., S. C. Podesta, B. Hatew, G. Klop, H. van Laar, A. Bannink, and J. Dijkstra. 2015. Effect of nitrogen fertilization rate and regrowth interval of grass herbage on methane emission of zero-grazing lactating dairy cows. *J. Dairy Sci.* 98:3383–3393.
- Watson, M., A. Wolf, and N. Wolf. 2003. Total nitrogen. Pages 18–24 in *Recommended Methods of Manure Analysis*. University of Wisconsin Extension publication no. A3769. J. Peters, ed. University of Wisconsin, Madison.
- Yan, T., R. E. Agnew, F. J. Gordon, and M. G. Porter. 2000. Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. *Livest. Prod. Sci.* 64:253–263.