The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows

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ABSTRACT

This study examined the effects of primary growth (PG) and regrowth (RG) timothy-meadow fescue silages harvested at 2 stages of growth on feed intake, cell wall digestion and ruminal passage kinetics in lactating dairy cows. Four dairy cows equipped with rumen cannulas were used in a study designed as a 4 × 4 Latin square with 21-d periods. The experimental silages were offered *ad libitum* with 8 kg/d of concentrate. Ruminal digestion and passage kinetics were assessed by the rumen evacuation technique. Silages of PG were on average more digestible than RG silages. The concentration of neutral detergent fiber (NDF) and indigestible NDF (iNDF) increased and the concentration of digestible organic matter in dry matter (DM) of silages decreased with advancing maturity in PG and RG. Cows consumed more feed DM, energy, and protein and produced more milk when fed PG diets rather than RG diets. Delaying the harvest decreased DM intake and milk production in PG and RG. There were no differences between PG and RG in rumen pH, ammonia N, or total volatile fatty acid concentrations. The intake of N, omasal canal flow of total nonammonia N and microbial N, excretion of N in feces, and ruminal true digestibility of N were higher for PG than for RG diets. The efficiency of microbial N synthesis was not different between PG and RG. Intake and omasal canal flow of organic matter, NDF, and potentially digestible NDF (pdNDF) were higher in PG than in RG. Whole-diet digestibility of organic matter, NDF, or pdNDF in the rumen or in the total tract was not different between PG and RG despite the higher digestibility of PG silages measured in sheep. Rumen pool sizes of crude protein and iNDF were lower for PG diets, whereas the pool size of pdNDF was higher for PG diets than for RG diets. The rate of passage of iNDF was higher for PG diets than for RG diets, with no difference between them in rate of digestion or pas-

sage of pdNDF. The lower milk production in cows fed regrowth grass silages compared with primary growth silages could be attributed to the lower silage DM intake potential. Chemical composition of the silages, rumen fill, digestion and passage kinetics of NDF, or the ratio of protein to energy in absorbed nutrients could not explain the differences in DM intake between silages made from primary and regrowth grass.

Key words: grass silage, dairy cow, maturity, regrowth

INTRODUCTION

Grass silage constitutes a large part of dairy cow rations in northern Europe and the intake of it contributes to the supply of digestible nutrients and subsequent milk production. The effects of growth stage on the digestibility and DMI of primary growth grass silages in dairy cows has been well documented (Rinne, 2000; Huhtanen et al., 2007). In contrast, the production responses to varying quality of regrowth silages have not been investigated widely, although a large proportion of the annual silage allowance is from regrowth silages. Milk production was lower when regrowth grass silages rather than primary growth grass silages were fed (Castle and Watson, 1970; Peoples and Gordon, 1989; Heikkilä et al., 1998). Intake of regrowth silages was often lower in these studies. However, in many experiments comparing primary growth with subsequent regrowths (second or third cut), only one silage per cut has been used, causing possible confounding effects of digestibility, DM and NDF concentration, or fermentation quality with cut effect. Because of these differences, the reasons for the lower milk yield and intake potential of silages harvested from regrowth versus primary growth grass remain unclear.

Intake and digestibility depend on the rates of NDF digestion and feed particle breakdown in the rumen and on the rate of digesta outflow from the rumen (Mertens, 1993). The physical limitation in the rumen could be a possible reason for the reduced DMI of cows consuming regrowth silage rather than primary growth silage.

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Rinne et al. (2002) documented a decrease in DMI and an increase in the passage rate and rumen pool size of NDF when the digestibility of primary growth grass silage decreased, suggesting that the importance of physical rumen fill in limiting feed intake increased as silage digestibility decreased. Imbalance between absorbed AA and energy could also limit DMI and milk production when regrowth silages are fed. It is well known that protein supplementation of dairy cows fed grass silage-based diets increases DMI and milk yield (Huhtanen et al., 2008). MacRae et al. (1985) concluded that reduced AA supply was the most probable reason for the lower efficiency of ME utilization for productive purposes when sheep were consuming regrowth grass rather than grass from primary growth.

The present study was designed to evaluate the effects of silage cut (primary growth vs. regrowth) and stage of maturity at harvest on digestibility, rumen fermentation, microbial protein synthesis, and rumen NDF digestion and passage kinetics in dairy cows, and to elucidate possible mechanisms explaining intake and production differences between the silages. To minimize confounding effects of other factors in studying cut effects, the silages were harvested at 2 different growth stages within the cut so that digestibilities overlapped. Furthermore, short wilting and a high level of formic acid application were used to minimize confounding effects of DM concentration and silage fermentation quality on the comparisons of cuts and growth stages. It was hypothesized that 1) grass silage intake and production responses are dependent on the cut (primary vs. regrowth), 2) grass silage intake and production responses are dependent on the growth stage, and 3) there are interactions between harvest and growth stage in grass silage intake and production responses. The results from the production trial using the same silages were reported previously (Kuoppala et al., 2008).

MATERIALS AND METHODS

Silages and Design of the Experiment

Two primary growth (PG) and 2 regrowth (RG) silages were prepared from mixed timothy (*Phleum pratense*) meadow fescue (*Festuca pratensis*) sward in 2002 in Jokioinen, Finland (N 60°48' E 23°27'). A detailed description on the preparation of the 4 silages was reported in our previous paper (Kuoppala et al., 2008). In brief, PG silages were harvested on June 5 at an early stage of growth and on June 17 at a late stage of growth. Silages from RG were harvested on July 29 at an early (primary growth cut on 17 June) stage and on August 12 at a late (primary growth cut on June 5) stage of growth. By harvesting the silages

of PG and RG at 2 growth stages, silage digestibility overlapped between PG and RG and it was possible to study the difference in response to the advancing maturity. The grass was cut with mower conditioner, wilted approximately 4 h, and harvested with a precision chop harvester. Grass was preserved with a formic-acid based additive containing 80% formic acid (Kemira Ltd., Helsinki, Finland) applied at a rate of 5.4 L/t in bunker silos (capacity 70 t). Botanical and morphological analysis was conducted from the samples collected from the areas adjacent to the silage harvest areas and the occurrence of leaf spot infections was evaluated by weekly sampling for 6 times of both regrowth areas, as reported in detail by Kuoppala et al. (2008).

The 4 silages—early-cut PG silage (PGE), late-cut PG silage (PGL), early-cut RG silage (RGE), and late-cut RG silage (RGL)—were fed in a 4 × 4 Latin square design to 4 Finnish Ayrshire cows fitted with rumen cannulas (10 cm i.d., Bar Diamond Inc., Parma, ID). Silages were supplemented with 8 kg/d concentrate (Raisio Feed Ltd., Raisio, Finland) comprising (% of fresh matter) barley (30), wheat bran (16.2), molassed sugar-beet pulp (15.0), rapeseed (canola) meal (13.0), wheat meal (6.3), oats (5.0), wheat molasses (5.0), soybean meal (2.0), maize gluten meal (2.3), rapeseed oil (1.0), and minerals and vitamins (4.2). The cows averaged 75 DIM (SD 11.9) and 617 kg of BW (SD 64.0) and produced, on average, 33 (SD 3.5) kg/d milk at the beginning of the study. Each experimental period lasted 21 d, consisting of a 16-d adaptation period and a 5-d sample collection period.

Cows had free access to silage during adaptation periods, but during sample collection periods, intake was restricted to 0.95 of that recorded during the adaptation period. Intake restriction was used to minimize between- and within-days variations in intake during sampling. Concentrates were offered in 2 equal meals at 0600 and 1800 h daily, and silage was offered 4 times daily at 0600, 0900, 1800, and 2000 h. Cows were milked at 0700 and 1700 h. All experimental procedures were approved by the local animal care and use committee.

Experimental Procedure

Representative samples of silage, concentrates, and feed refusals (if these exceeded 300 g/d) were collected daily during the sample collection period and stored at −20°C. At the end of the experiment, samples were thawed, mixed, and submitted for chemical analysis. Fresh silage samples were analyzed for pH and concentrations of VFA, lactic acid, water-soluble carbohydrates (WSC), ammonia N, and water-soluble N. The content of DM was determined by drying at 105°C for 20 h and that of OM by ashing at 600°C for 2 h. Oven DM

content of silages was corrected for the loss of volatiles. The concentration of NDF was determined according to Van Soest et al. (1991) using Na-sulfite, without amylase for forages and reported ash-free. Permanganate lignin was determined according to Robertson and Van Soest (1981). Methods of analyses were reported in detail by Kuoppala et al. (2008).

The concentration of CP was analyzed by the Dumas method using a Leco FP 428 nitrogen analyzer (Leco Corp., St Joseph, MI). The concentration of indigestible NDF (**iNDF**) was determined by 12-d ruminal incubation in the rumen of dairy cows fed a forage-rich diet, using nylon bags with a pore size of 17 μm (Huhtanen et al., 1994) and expressed on an ash-free basis. Apparent *in vivo* digestibility of the silages was measured simultaneously with male sheep by total fecal collection, as reported in Kuoppala et al. (2008).

Milk samples were taken on 4 consecutive milkings in the last week of each period and analyzed by using an infrared analyzer (MilkoScan 605, Foss Electric, Hillerød, Denmark) for fat, protein, and lactose. Milk composition was calculated as the weighted mean of the analyses. Milk samples for urea determination were taken on 2 milkings. Concentration of urea was calculated from the difference in the concentration of ammonia N between the unhydrolyzed sample and that hydrolyzed with urease. Ammonia N was determined by using the method of McCullough (1967).

A detailed description of the total collection of feces and urine on d 18 to 21, as well as the omasal sampling technique used to obtain samples from the omasal canal to determine total and ruminal digestibility of diets, respectively, is given by Ahvenjärvi et al. (2000). In brief, 2 external markers, LiCoEDTA and Yb-acetate, were used for the liquid and small particle phases, respectively. An internal marker, iNDF, was used as a marker for large particle phase. Priming doses of LiCoEDTA (18 g) and Yb (7.5 g) were given 60 h before the first sampling via the rumen cannula to facilitate a rapid equilibrium of marker concentrations in the rumen. Thereafter, a continuous infusion of LiCoEDTA (12 g/d) and Yb-acetate (5.8 g of Yb/d), dissolved in 6 L of distilled water, was administered until the end of each period, using a peristaltic pump (Watson-Marlow 502 S, Falmouth, UK). Omasal canal samples of 500 mL were collected on d 18 to 21 three times daily, starting at 0600, 1000, and 1400 h on d 18 and advancing by 1 h each day so that samples represented each hour during the 12-h daytime feeding cycle. The samples were frozen immediately after sampling and stored at -20°C until being thawed at room temperature and pooled over the sampling times to form a single sample per cow per period. The fractionation of the samples into large particles, small particles, and liquid phase, and

the analysis of the chemical composition of fecal and digesta samples, as well as markers used, were described in detail previously (Ahvenjärvi et al., 2002).

The flow of microbial N from the rumen was determined using 17.5 g/d of ammonium sulfate (Isotec Inc., Miamisburg, OH) with 10% enrichment of ^{15}N (371 mg of $^{15}\text{N}/\text{d}$) as a marker. Infusion of the marker dissolved into the infusion solution of ytterbium started 48 h before the first sampling. Before the beginning of the marker infusion, samples of rumen contents of each cow were taken on d 11 during the first period to determine the background abundance of ^{15}N . Samples for bacterial separation (500 mL) were taken from reticular digesta at 1500, 1200, 0900, and 0600 h on d 18, 19, 20, and 21, respectively. Immediately after collection, samples were centrifuged and the supernatant was decanted through 2 layers of cheesecloth. Differential centrifugation of these samples is described in detail by Ahvenjärvi et al. (2000). Measurement of ^{15}N enrichment in bacterial pellets and omasal samples was similar to that reported by Ahvenjärvi et al. (2002).

To determine rumen pool sizes, rumen evacuations were conducted on d 13 (before morning feeding) and d 15 (6 h after morning feeding). The times were chosen to represent the minimum and maximum rumen fill. Rumen contents were emptied manually through the cannula, kept warm in a water bath, mixed thoroughly, weighed, and sampled (2–3 kg of fresh weight). After sampling, the total digesta were immediately returned into the rumen.

To assess ruminal fermentation, 100- to 150-mL liquid samples were obtained on d 21 before the morning meal, and 1.5, 3, 4.5, 6, 7.5, 9 and 10.5 h thereafter via the rumen cannula using a perforated plastic tube. The samples were immediately measured for pH and then prepared for the determination of ammonia-N and VFA, as described by Ahvenjärvi et al. (2002). Samples were stored at -20°C before analysis. For the VFA analysis, samples were pooled over the sampling times to provide one sample per each cow per period. For statistical analysis, pH and ammonia N mean values over the sampling times were used.

Calculations and Statistical Analyses

Concentrations of ME and MP of feeds were calculated according to Finnish Feed Tables (MTT, 2006). Digesta flow into the omasal canal was calculated using a triple-marker method (France and Siddons, 1986). Organic matter truly digested in the rumen was calculated based on microbial N to OM ratio determined from bacterial samples. Enrichment of ^{15}N in excess of background levels in bacterial and reconstituted omasal canal digesta samples was calculated by difference to

blank samples collected before the beginning of the experiment (0.3678 and 0.3690 ^{15}N -atom %, respectively). The omasal canal flow of NAN was calculated by difference between total N and ammonia N flows. Ammonia N of omasal digesta was 0.7% of total N flow. Ash-free neutral detergent solubles (**NDS**) were calculated as the difference between OM and NDF. Potentially digestible NDF (**pdNDF**) was calculated as the difference between NDF and iNDF. Rumen pool sizes were calculated as the mean of the 2 evacuation times and are assumed to represent the average rumen fill. Rate of intake (k_i), rate of passage (k_p), and rate of digestion (k_d) were calculated (Robinson et al., 1987) for pdNDF and k_p for iNDF as follows:

$$k_i = 1/24 \times (\text{intake, kg/d})/(\text{rumen pool size, kg});$$

$$k_p = 1/24 \times (\text{omasal canal flow, kg/d})/(\text{rumen pool size, kg});$$

$$k_d = k_i - k_p.$$

Experimental data were subjected to ANOVA using the general linear model procedure (PROC GLM) of SAS software for Windows version 9.1.3 (SAS Institute Inc., Cary, NC) using the model

$$Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk},$$

where Y_{ijk} = dependent variable, μ is the overall mean, A_i is the fixed cow effect, P_j is the period effect, D_k is the diet effect, and e_{ijk} is the residual effect. Main effects of cut and growth stage and their interaction were analyzed using orthogonal contrasts as follows: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut $\times$ growth stage interaction. Least squares means are presented in tables, and statistical significance refers to $P \leq 0.05$.

RESULTS

Chemical composition of the experimental feeds is given in Table 1. All silages were of good fermentation quality, as evidenced by the low pH and concentration of ammonia N in total N. The concentration of total fermentation acids in silages was moderate (between 4.3 and 8.7% of DM), indicating restricted fermentation. For both early harvested silages, WSC was fermented to a greater extent to lactic acid and VFA compared with the late harvested silages. The high WSC concentration in RGL may have been caused, in part, by the higher DM concentration. The lower concentration of DM in

RGE compared with the other silages was caused by rainy weather conditions before the harvest.

Primary growth silages had a lower mean concentration of ash than did RG silages, resulting in a higher concentration of digestible OM in DM (**DOM**) at the same OM digestibility (**OMD**). Silages of PG were, on average, more digestible than RG silages (OMD 73.0 vs. 70.3%, respectively). The concentration of NDF and iNDF increased and DOM of silages decreased with advancing maturity in PG and RG silages, but differences between PG silages were greater than between RG silages. Concentrations of NDF and iNDF of RG silages were intermediate to the values of PG silages.

Mean intakes of DM, ME, and MP, and yields of milk and ECM were higher ($P < 0.01$) for PG diets than for RG diets (Table 2). Delaying the harvest decreased both DMI and milk production ($P < 0.05$). Milk protein concentration tended to be higher in PG than in RG diets, with no difference in fat or lactose concentrations between PG and RG. Delaying the harvest decreased milk protein concentration for PG diets, with no change for RG diets (interaction $P < 0.05$). The concentration of MUN was higher ($P = 0.05$) for RG than for PG diets. Delaying the harvest decreased MUN, with a more profound reduction for the RG diets than for the PG diets (interaction $P < 0.05$).

There were no differences between PG and RG in rumen pH, ammonia N, or total VFA concentrations (Table 3). Delaying the harvest decreased ($P < 0.05$) rumen ammonia-N and total VFA concentrations, and the decrease of ammonia-N was greater for RG ($P < 0.05$) than for PG diets. The molar proportion of acetate was higher for RG than for PG diets, with no effect of maturity in RG; however, delaying the harvest in PG increased the molar proportion of acetate (interaction $P < 0.05$). The proportion of propionate decreased in PG with maturity but increased in RG (interaction $P = 0.01$). The proportions of the other acids were higher ($P < 0.05$) for PG than for RG diets. The acetate + butyrate to propionate ratio increased for PG diets but decreased for RG diets with advancing maturity (interaction $P < 0.01$).

The intake of N, omasal canal flow of total and microbial NAN, excretion of N in feces, and ruminal true digestibility of N were higher ($P < 0.05$) for PG than for RG diets (Table 4). Nitrogen intake and flow reflected the CP concentration and intake of silages, which were lower for RG compared with PG. The differences in omasal canal NAN flow were mainly explained by differences in microbial NAN flow, because no differences in nonmicrobial NAN flow between PG and RG were observed. The efficiency of microbial N synthesis was also not affected by the cut. Delaying the harvest de-

Table 1. Chemical composition of the grass silages cut at early and late stages of primary growth and regrowth periods and the concentrate

Item	Primary growth		Regrowth		Concentrate
	Early	Late	Early	Late	
Harvest date in 2002	5 June	17 June	29 July	12 August	
Previous harvest date	—	—	17 June	5 June	
DM, %	28.3	28.3	22.7	33.4	88.1
In DM, %					
Ash	8.2	6.8	9.8	9.0	7.8
CP	15.5	12.7	15.7	11.6	17.8
NDF	49.8	58.9	51.3	53.8	24.6
iNDF ¹	5.0	9.7	6.0	9.3	5.1
pdNDF ²	44.8	49.2	45.3	44.3	19.5
Lignin	2.3	2.7	2.2	2.8	1.3
Acetic acid	1.74	1.31	1.55	1.12	
Propionic acid	0.015	0.010	0.015	0.007	
Butyric acid	0.130	0.048	0.091	0.044	
Lactic acid	6.84	4.07	6.50	3.09	
Water-soluble carbohydrates	3.06	8.61	2.39	13.66	
pH	3.97	4.22	3.92	4.30	
Ammonia-N, % of N	4.25	5.68	5.61	6.70	
Soluble N, % of N	65.5	61.6	53.5	52.0	
Cell wall characteristics, %					
iNDF/NDF	10.1	16.5	11.7	17.3	20.7
Lignin/NDF	4.5	4.6	4.3	5.2	5.3
Lignin/iNDF	45.0	27.7	36.7	29.9	25.5
OMD, ³ %	76.7	69.2	73.4	67.3	
DOM, ⁴ % of DM	70.4	64.4	66.4	60.9	
ME, MJ/kg of DM	11.3	10.3	10.6	9.7	12.4
Silage DMI index ⁵	107	98	91	93	

¹Indigestible NDF.²Potentially digestible NDF.³Digestibility of OM determined in vivo with sheep.⁴Digestible OM in DM.⁵Silage DMI index according to Huhtanen et al. (2007).

creased ($P < 0.05$) N intake, omasal canal flow of NAN and nonmicrobial NAN, and N digestibility in the rumen and in the total tract. The efficiency of microbial protein synthesis increased as the harvest was delayed ($P < 0.01$). The decrease of omasal canal NAN flow, caused by delaying the harvest, was smaller when RG

rather than PG diets were fed (interaction $P < 0.05$). Excretion of N in the feces increased in RG (interaction $P < 0.05$) with advancing maturity, but not in PG.

Intake of OM, NDF, pdNDF, and NDS and omasal canal flow of OM and NDF were higher ($P < 0.05$) in PG than in RG (Table 5). There were no differ-

Table 2. The effects of harvesting strategy on daily feed and nutrient intake and milk production of dairy cows

Item	Primary growth		Regrowth		SEM	Contrast, ¹ P -value		
	Early	Late	Early	Late		C1	C2	C3
Intake per day								
Silage DM, kg	13.8	12.2	11.2	10.6	0.38	<0.01	0.02	0.21
Total DM, kg	20.9	19.2	18.3	17.6	0.38	<0.01	0.02	0.21
ME, MJ	244	213	206	190	4.12	<0.01	<0.01	0.11
MP, ² g	1,947	1,710	1,681	1,541	30.6	<0.01	<0.01	0.16
Milk yield, kg/d	27.6	25.1	24.9	23.7	0.47	<0.01	<0.01	0.20
ECM yield, kg/d	27.2	23.9	23.5	22.5	0.55	<0.01	<0.01	0.08
Milk composition, %								
Fat	3.85	3.70	3.70	3.69	0.079	0.35	0.35	0.43
Protein	3.33	3.20	3.12	3.15	0.024	<0.01	0.10	0.02
Lactose	4.78	4.74	4.74	4.74	0.034	0.56	0.56	0.48
MUN, mg/dL	9.90	8.85	12.4	8.37	0.417	0.05	<0.01	0.01
kg of ECM/kg of DMI	1.31	1.24	1.29	1.28	0.039	0.82	0.40	0.50

¹Orthogonal contrasts: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut $\times$ growth stage interaction.²MTT (2006).

Table 3. The effects of harvesting strategy on rumen fermentation of dairy cows

Item	Primary growth		Regrowth		SEM	Contrast, ¹ <i>P</i> -value		
	Early	Late	Early	Late		C1	C2	C3
pH	6.46	6.62	6.50	6.57	0.078	0.96	0.18	0.60
Ammonia, mg/dL	8.21	8.06	9.56	4.87	0.685	0.23	0.01	0.02
Total VFA, mmol/L	108.0	100.0	109.5	99.9	3.35	0.84	0.04	0.82
In total VFA, mmol/mol								
Acetate	631	653	654	656	3.1	0.01	0.01	0.02
Propionate	194	189	192	201	1.7	0.03	0.36	0.01
Butyrate	134	123	121	117	2.5	0.01	0.03	0.24
Isobutyrate	7.8	7.3	7.9	5.6	0.21	0.01	<0.01	0.01
Valerate	15.8	12.9	11.9	10.5	0.50	<0.01	0.01	0.17
Isovalerate	10.9	9.5	9.7	6.2	0.58	0.01	0.01	0.13
Caproate	6.8	5.0	3.8	4.1	0.51	0.01	0.20	0.08
(Acetate + butyrate)/propionate	3.93	4.10	4.03	3.85	0.041	0.11	0.93	0.01

¹Orthogonal contrasts: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut × growth stage interaction.

ences between PG and RG in ruminal or total-tract digestibility of OM, NDF, and pdNDF. Delaying the harvest decreased the intake of OM and NDS ($P < 0.05$), the decrease of NDS intake being smaller for RG than PG diets (interaction $P < 0.01$). Omasal canal flow increased with advancing maturity for OM, NDF, and pdNDF ($P < 0.01$). The apparent digestibility in the rumen and in the total tract was lower in the later harvests of PG and RG silages for OM, NDF, and pdNDF ($P < 0.05$).

Rumen pool sizes of CP and iNDF were lower ($P < 0.01$) for PG diets, whereas the pool size of pdNDF was higher ($P < 0.05$) for PG diets than for RG diets (Table 6). The ratios of iNDF to pdNDF in the rumen contents (0.36 vs. 0.46 for PG and RG, respectively) and in feces (0.62 vs. 0.72 for PG and RG, respectively) were higher ($P < 0.01$) for RG diets than for PG diets, with no difference between them in the omasal canal. The k_p of iNDF was higher ($P < 0.01$) for PG diets than for RG diets, with no difference between PG and RG in k_d or k_p of pdNDF. Delaying the harvest increased rumen pool sizes of OM, CP, NDF, and iNDF ($P < 0.05$), the

ratio of iNDF to pdNDF in the rumen contents and in feces ($P < 0.01$), and k_p of iNDF ($P < 0.01$). Delaying the harvest decreased k_d of pdNDF ($P < 0.01$) but increased k_p of pdNDF ($P < 0.05$).

DISCUSSION

Composition and Digestibility of Silages

A lower concentration of CP and NDF and higher concentration of iNDF as well as lower OMD in RG than in PG silages seem to be typical trends as they were also observed by Huhtanen et al. (2006b) using a larger data set. The range in chemical composition due to delaying the harvest was smaller for RG silages than for PG silages except for CP and lignin. The mean value of iNDF was lower for both PG and RG, whereas the mean value of OMD of PG was on the same level and that of RG higher than the average values reported by Huhtanen et al. (2006b) for silages made from primary growth and regrowth of grass.

The ratios of iNDF and lignin to NDF were higher in RG silages compared with PG silages, indicating a

Table 4. The effects of harvesting strategy on N digestion of dairy cows

Item	Primary growth		Regrowth		SEM	Contrast, ¹ <i>P</i> -value		
	Early	Late	Early	Late		C1	C2	C3
N intake, g/d	544	451	482	397	12.9	0.01	<0.01	0.75
Omasal canal NAN flow, g/d	490	442	430	417	6.5	<0.01	0.01	0.04
Microbial NAN	293	268	238	234	6.3	<0.01	0.06	0.15
Nonmicrobial NAN	197	174	192	183	5.6	0.71	0.03	0.28
N excreted in feces, g/d	157	148	140	147	3.0	0.03	0.78	0.03
N digestibility, %								
Ruminal, apparent	9.0	1.3	10.0	−5.6	2.23	0.23	<0.01	0.13
Ruminal, true	63.6	61.4	60.1	53.8	1.57	0.01	0.03	0.24
Total tract, apparent	71.1	67.3	71.0	62.9	1.05	0.08	<0.01	0.08
Microbial N, g/kg of OMTDR ²	20.3	22.7	19.7	23.1	0.85	0.93	0.02	0.60

¹Orthogonal contrasts: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut × growth stage interaction.

²OM truly digested in the rumen.

Table 5. The effects of harvesting strategy on fiber digestion of dairy cows

Item	Primary growth		Regrowth		SEM	Contrast, ¹ <i>P</i> -value		
	Early	Late	Early	Late		C1	C2	C3
Intake, kg/d								
OM	19.2	17.9	16.6	16.1	0.347	<0.01	0.04	0.25
NDF	8.63	8.91	7.48	7.42	0.219	<0.01	0.64	0.47
pdNDF ²	7.58	7.37	6.45	6.07	0.193	<0.01	0.18	0.68
NDS ³	10.6	8.95	9.13	8.72	0.143	<0.01	<0.01	<0.01
iNDF ⁴	1.05	1.55	1.03	1.35	0.034	0.02	<0.01	0.05
Omasal canal flow, kg/d								
OM	7.90	8.90	7.10	7.90	0.123	<0.01	<0.01	0.43
NDF	2.90	4.36	2.87	3.61	0.114	0.01	<0.01	0.02
pdNDF	1.88	2.92	1.96	2.35	0.111	0.07	<0.01	0.03
Digestibility in rumen, %								
OM, apparent	59.0	50.1	57.5	50.9	0.85	0.69	<0.01	0.23
OM, true	75.0	66.1	72.6	63.5	2.24	0.30	<0.01	0.96
NDF	66.1	51.1	61.6	51.5	1.22	0.13	<0.01	0.09
pdNDF	74.9	60.4	69.5	61.3	1.38	0.16	<0.01	0.06
Digestibility in total tract, %								
OM, apparent	75.4	69.7	75.8	70.1	0.542	0.46	<0.01	0.96
NDF	65.9	58.9	68.6	57.9	1.16	0.49	<0.01	0.17
pdNDF	74.6	69.8	77.6	69.3	1.02	0.28	<0.01	0.13

¹Orthogonal contrasts: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut × growth stage interaction.

²pdNDF = potentially digestible NDF = NDF – iNDF.

³NDS = neutral detergent solubles = OM – NDF.

⁴iNDF = indigestible NDF.

less favorable composition of fiber in RG silages, which may have contributed to decreased digestibility. In the present study, RGE was harvested after a growing period of 42 d, and such a short growing time in PG usually causes high digestibility under Nordic climatic conditions. However, the DOM of RGE was lower than that of PGE. Lower digestibility of RG silages has

been observed previously (Castle and Watson, 1970; Khalili et al., 2005), and one reason for that may be the higher mean temperatures that typically occur during the regrowth period compared with the primary growth period. High temperature increases lignification and promotes higher metabolic activity in plants (Van Soest, 1994). Wilson et al. (1991) concluded that high

Table 6. The effects of harvesting strategy on rumen pool size, passage and digestion kinetics, and the ratio of indigestible NDF (iNDF) and potentially digestible NDF (pdNDF) in the rumen, omasal canal, and feces

Item	Primary growth		Regrowth		SEM	Contrast, ¹ <i>P</i> -value		
	Early	Late	Early	Late		C1	C2	C3
Volume of rumen contents, L	99.3	107.8	108.7	104.2	3.00	0.37	0.54	0.07
Rumen contents, kg								
Fresh matter	86.5	94.7	93.9	94.0	2.33	0.19	0.12	0.13
DM	11.3	12.6	12.0	12.3	0.38	0.59	0.08	0.24
OM	10.3	11.6	10.7	11.1	0.35	0.90	0.05	0.22
CP	2.20	2.04	2.56	2.15	0.053	<0.01	<0.01	0.06
NDF	6.65	8.11	6.52	7.52	0.252	0.20	<0.01	0.39
pdNDF ²	5.10	5.77	4.64	4.98	0.252	0.05	0.09	0.53
iNDF	1.55	2.34	1.88	2.54	0.046	<0.01	<0.01	0.20
iNDF/pdNDF in								
Rumen	0.302	0.408	0.406	0.512	0.0223	<0.01	<0.01	0.99
Omasal canal	0.565	0.531	0.529	0.575	0.0212	0.87	0.78	0.11
Feces	0.550	0.698	0.715	0.725	0.0196	<0.01	<0.01	0.01
pdNDF k_d , ³ %/h	4.65	3.28	4.13	3.15	0.240	0.22	<0.01	0.44
pdNDF k_p , ³ %/h	1.54	2.14	1.81	2.01	0.122	0.60	0.02	0.15
iNDF k_p , %/h	2.90	2.79	2.34	2.24	0.086	<0.01	0.28	0.98

¹Orthogonal contrasts: C1 = cut effect (primary growth vs. regrowth), C2 = growth stage, C3 = cut × growth stage interaction.

²pdNDF = NDF – iNDF.

³ k_p = rate of passage; k_d = rate of digestion.

temperature during growth increased intensity of lignification of the existing lignified cells. In the present study, the mean daily temperature was considerably higher during regrowth (June–July/August, 17.2°C) than during spring growth of PG grass (April–June, 12.0°C), as reported by Kuoppala et al. (2008), which may have affected the pattern of lignification.

Grass regrowth contains more leaves relative to stems compared with primary growth (Castle and Watson, 1970; Rinne and Nykänen, 2000; Kuoppala et al., 2003), indicating more vegetative material in the regrowth. A high proportion of leaves is generally associated with a higher digestibility and intake of forages within a cut, but this may not be the case when forages from different cuts are compared. Despite the higher leaf to stem ratio of RG grass (Kuoppala et al., 2008), digestibility of RG silages was lower compared with that of PG silages. Pakarinen et al. (2008) found an overall positive correlation between leaf proportion and DOM over 2 yr and cuts, but no such correlation was found in RG when the cuts were analyzed separately.

Further differences between PG and RG were the increased heterogeneity of plant material in RG, indicated by higher proportions of other plant species (weeds) and dead tissues, and the higher proportion of meadow fescue than timothy in RG compared with PG (Kuoppala et al., 2008), which may have been responsible for the lower digestibility and intake responses. Also, the incidence of fungal grass disease found in autumn grass (Heikkilä et al., 1998; Kuoppala et al., 2008) may contribute to reduced DMI. *In vitro* digestibility of dead grass material has been shown to be very low and thus a high proportion of it may decrease the digestibility of whole herbage yield (Pakarinen et al., 2008). Regrowth grass herbages also contain more waxes and cutins in cell soluble matter (Van Soest, 1994), which have a low availability in the animal and may cause the lower true digestibility of NDS for RG silages, compared with PG silages, as suggested by Huhtanen et al. (2006b).

Intake and Milk Production

Differences in intake and milk production found in the present metabolism study were in agreement with those in a production trial with the same silages (Kuoppala et al., 2008). The maturity and cut effects on milk production are discussed in detail in that paper.

An increase in digestibility has consistently increased silage DMI and milk yield with primary growth grass silages (Rinne, 2000; Huhtanen et al., 2007), but between PG and RG the relationship seems to be different. The higher average digestibility of PG silages allowed a higher intake for PG diets compared with RG diets. However, RGE had a higher digestibility and

lower concentration of NDF than PGL, but despite that, cows consumed more PGL. MacRae et al. (1985) found a lower efficiency of ME utilization for productive purposes when sheep were consuming regrowth grass rather than grass from primary growth; the authors concluded that reduced AA supply was the most probable reason for that finding. In the present study, the efficiency of energy utilization for milk production did not differ between PG and RG (0.112 vs. 0.116 kg of ECM/MJ of ME for PG and RG, respectively), in accordance with Peoples and Gordon (1989) and Heikkilä et al. (1998). Thus, milk production reflected the DMI and the subsequent ME intake.

Huhtanen et al. (2007) reported, based on a meta-analysis of 46 diets, that the relative silage DMI index predicts lower intakes of regrowth grass silages compared with primary growth grass silages of similar digestibility, DM and fiber concentration, and extent of *in-silo* fermentation. In the silage DMI index, the coefficient of the proportion of RG silage (0–1) was 0.44 (DMI of RG silage was 0.44 kg/d lower than that of comparable PG silage). However, this effect was rather variable, suggesting variation between studies in factors that are not routinely analyzed; for example, abundance of plant diseases that can influence palatability attributes of the feed (Huhtanen et al., 2007).

The decrease in silage DMI was, on average, 0.175 kg (Huhtanen et al., 2007) when the DOM of PG grass silage decreased by 1 percentage unit. Compared with that, the intake response to increased silage digestibility was higher with PG, but lower with RG (0.28 kg and 0.11 kg per 1 percentage unit of DOM for PG and RG, respectively). The response in milk yield was 0.3 to 0.5 kg per 1 percentage unit of DOM (Rinne, 2000) in PG. In the present study, the response was within that range in PG but smaller in RG (0.42 and 0.21 kg per 1 percentage unit of DOM for PG and RG, respectively). In a production trial (Kuoppala et al., 2008), ECM yield responses were much more closely related to energy intake than silage digestibility, probably because of differences in silage concentrations of DM and fermentation acids, which also influence intake. The effect of delaying the harvest in RG on milk production seems not to be as straightforward and clear as in PG, probably reflecting smaller intake responses to improved digestibility. Delaying the harvest in RG has not always decreased DOM as constantly as in PG, or the cows have eaten more silage in spite of the lower digestibility. In the experiment of Gordon (1980), dairy cows consumed less silage and produced less milk when the regrowth interval was increased from 5 to 9 wk and DOM decreased from 73 to 62%, giving a decrease of 0.25 kg in silage DMI and 0.33 kg in milk yield per 1 percentage unit decrease in DOM.

Ruminal OM and N Metabolism

Higher acetate and lower butyrate proportion in total VFA in the rumen for RG diets, compared with PG diets, was in accordance with Bertilsson and Murphy (2003) and Korhonen (2003). In general, a decrease in the concentration of total VFA and ammonia N, as well as an increase in the molar proportion of acetate and decrease in butyrate, with delayed harvest, was in accordance with earlier results (Rinne et al., 2002; Harrison et al., 2003). The difference between the cuts was mainly the different effect of maturity on the proportion of acetate (increase in PG and no effect in RG) and propionate (decrease in PG and increase in RG) and on the acetate + butyrate ratio to propionate, which increased in PG but decreased in RG.

The intake of N was lower in RG than in PG. In general, N flow from the rumen is related more closely to intake of DOM, which dictates microbial N synthesis, because rumen-degradable grass silage N in excess of microbial requirements leaves the rumen as ammonia N (flow and absorption), and omasal canal N flow is very close to NAN flow. The average whole-diet CP concentrations were 16.3, 14.7, 16.5, and 14.1% of DM for PGE, PGL, RGE, and RGL, respectively. There might have been a slight deficiency of rumen-degradable N for the RGL diet because the average ammonia-N concentration and the minimum values during the feeding interval (<2.8 mg/dL, data not shown) in the rumen of cows consuming RGL were low. Rumen ammonia-N concentration for RGL approached the values reported by Satter and Slyter (1974) required for optimal microbial protein synthesis (5.0 mg/dL) and was lower than the suggested minimum level for optimal fiber digestion (8.0 mg/dL; Hoover, 1986). On the other hand, the observed average MUN for RGL was >7.4 mg/dL, which has been suggested to indicate an adequacy of rumen-degradable protein in the diet (Nousiainen, 2004). The average omasal canal NAN flow exceeded N intake for RGL consistently with low ruminal ammonia-N concentration, suggesting net utilization of recycled N. High MUN was observed with diet RGE, but the reasons for this remain unclear because rumen ammonia-N concentration, ruminal N losses, and efficiency of N utilization were in line with the differences in dietary CP concentration.

Lower total NAN flow entering the lower tract with RG diets compared with PG diets was mainly explained by lower microbial NAN flow. The contribution of microbial NAN to the total NAN was, on average, lower for RG (56%) than for PG diets (60%). No difference between cuts was found in nonmicrobial NAN flow, despite the lower N digestibility in the rumen with RG diets. The average efficiency of microbial protein

synthesis was not different between cuts; therefore, the lower NAN flow of RG diets was caused by the lower DMI and lower amount of OM digested in the rumen.

Increased silage DM intake in response to protein supplementation in dairy cows (Huhtanen et al., 2008) suggests that improved AA to ME balance expressed as MP/ME at the tissue level could be a mechanism influencing intake. However, that seems unlikely in the present study because the ratio of MP intake to ME intake was not different between PG and RG diets (8.0 vs. 8.1 g of MP/MJ of ME for PG and RG, respectively). Also, the NAN flow per digestible OM intake was not affected by the cut.

Fiber Digestion and Passage Kinetics

Digestibility is one of the most important factors affecting silage intake (Huhtanen et al., 2007). Most of the variation in diet digestibility in cows fed grass silage-based diets is related to dietary NDF concentration and NDF digestibility (Huhtanen et al., 2009). Digestibility of OM or NDF in the present study does not appear to explain the differences of DMI for PG and RG diets because no differences were found between PG and RG in rumen or total-tract digestibility. Intake and omasal canal flow of NDF and pdNDF reflected total DMI and NDF concentrations.

Lower k_p of iNDF for RG diets compared with PG diets resulted in a larger iNDF pool size and a greater proportion of iNDF of total NDF in the rumen contents. No difference was found between cuts in pool sizes of fresh matter, DM, or NDF, but the largest pool sizes of all diets were observed for PGL, with the exception of iNDF pool size, which was largest for RGL. Given that the numerical value of k_p of iNDF was lowest for RGL, it can be speculated that there was no need for faster passage because the fill was not limiting, as indicated by the smaller pool size of NDF compared with PGL. Thus, if the pool size of NDF indicates the physical fill, it can be concluded that the pool size of NDF or iNDF has not limited DMI for RG diets, and physical constraints limiting DMI might have existed only for PGL.

Delay of harvest increased the pool size of NDF, pdNDF, and iNDF in PG and RG, which was in accordance with earlier results in primary growth (Rinne et al., 2002). van Vuuren et al. (1999) compared ryegrass regrowth silages with regrowth interval of 21 d versus 37 d and found no effect of regrowth interval on ruminal pool sizes of DM and OM. Feeding more mature forage increased the ruminal pool size of NDF but not the turnover rate, suggesting that the increase in pool size was due to higher NDF intake. The intake of NDF explained 92% of variation in k_p of iNDF in the present

study, with a slope of 0.44. Huhtanen et al. (2006a) reported a linear relationship between NDF intake and k_p of iNDF with R^2 of 0.86 and a slope of 0.27.

The k_p of iNDF in the present study was not affected by a delay of harvest, which agreed with the findings of Lund et al. (2007) but not with those of Rinne et al. (2002). The decrease in k_d and increase in k_p of pdNDF, caused by the delay in harvest in the present study, was in accordance with Rinne et al. (2002), and explained, in part, the lower digestibility of pdNDF due to the delay of harvest.

Owens et al. (2008b) reported, from an experiment with autumn grass, that an increase of regrowth interval from 35 to 45 d did not affect k_d of pdNDF or k_p of iNDF of beef cattle. Pool sizes of DM, OM, NDF, or pdNDF were also not affected. Using grass harvested in spring with a regrowth interval of 28 and 38 d, they found that prolonging the regrowth interval increased the pool size of NDF and pdNDF and decreased k_d of pdNDF, with no effect on k_p of iNDF (Owens et al., 2008a).

CONCLUSIONS

The lower milk production in cows fed regrowth grass silages compared with primary growth silages could be attributed to the lower silage DM intake. The chemical composition of the silages, rumen fill, digestion and passage kinetics of NDF, or the ratio of MP to ME in absorbed nutrients could not explain the differences in DM intake between silages made from primary and regrowth grass. The efficiency of microbial N synthesis and the omasal canal flow of nonmicrobial NAN were unchanged between cuts. Based on the current findings and previous work, we recommend that silage prepared from regrowth grass should preferably be directed to groups of livestock other than high-yielding dairy cows in early lactation.

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