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Fiber digestibility and protein value of pulp silage for lactating dairy cows: Effects of wet fractionation by screw pressing of perennial ryegrass

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ABSTRACT

The aim of the study was to investigate the effects of substituting silage of chopped grass with pulp silage of grass fractionated once or twice in a biorefinery using a screw press on fiber kinetics, protein value, and production of CH_4 in dairy cows. Six lactating multiparous Holstein cows in mid-lactation (176 ± 93 d in milk), cannulated in the rumen, duodenum, and ileum, were used in an incomplete 6 × 4 Latin square design with a 2 × 3 factorial arrangement of treatments. Perennial ryegrass was harvested in third regrowth from the same field at early and late developmental stage (35 and 44 d of regrowth, respectively) and subjected to 1 of 3 types of processing within each developmental stage. Grass was either harvested for normal silage making (mowed, wilted, chopped, and ensiled), or harvested fresh and fractionated using a screw press. Half of the pulp from the first fractionation was ensiled, whereas the other half of the pulp was rehydrated, fractionated a second time, and pulp hereof was ensiled. The grass and pulp silages were used with concentrates (65:35 forage to concentrate ratio) to make total mixed rations (TMR) based on either silage of chopped grass (GS), pulp silage of grass fractionated once (1×P), or pulp silage of grass fractionated twice (2×P), harvested either at early (E) or late (L) developmental stage resulting in 6 different TMR treatments (EGS, E1×P, E2×P, LGS, L1×P, L2×P). The TMR were fed for ad libitum intake and samples of intestinal digesta and feces were collected for determination of digestibility. The effect of processing on ash-free neutral detergent fiber (aNDFom) concentration in silages depended on developmental stage, but showed that within each developmental stage, pulp silage of grass fractionated twice had higher aNDFom concentration than pulp silage of grass fractionated once and silage of chopped grass. The 2×P resulted in lower (14.9 ± 0.55 vs. 17.5 ± 0.54 kg/d) dry matter intake (DMI) compared with GS. The effects of processing and developmental stage interacted such that apparent total-tract aNDFom digestibility was higher (784 ± 13 vs. 715 ± 13 g/kg) for L2×P compared with LGS, whereas no difference was found between E2×P and EGS. Moreover, the protein value was higher (106 ± 5 vs. 92 ± 5 g AA digested in the small intestine/kg of DMI) for 2×P compared with GS. Unexpectedly, processing had no effect on fractional rate of digestion of digestible aNDFom or CH_4 yield (L/kg of DMI), whereas feeding forages harvested at early compared with late developmental stage resulted in lower CH_4 yield. Feeding pulp silage of grass fractionated once generally yielded results intermediate to cows fed silage of chopped grass and pulp silage of grass fractionated twice. This study showed that pulp silage of fractionated grass could serve as feed for dairy cows because the fiber digestibility and protein value improved, but further research investigating effects of physical processing of forage on fiber kinetics is required.

Key words: fiber kinetic, forage, methane, perennial ryegrass, ruminant

INTRODUCTION

In a biorefinery, green forages are fractionated into a fibrous pulp, which can be used in ruminant nutrition, and a protein-rich liquid, suited for meeting the increased global demand for sustainable animal-based protein. Green forages such as grass, clover, and lucerne are used for fractionation because they provide high yields of CP per hectare (Wilkins and Jones, 2000) and the CP can be used efficiently by pigs and poultry if it is fractionated from the fibrous part during biorefining (Pirie, 1978). However, on DM basis, extraction of protein from forages still yields a quantitatively dominating side-stream of fibrous pulp because approximately 65% of DM is recovered in the pulp (Damborg et al., 2020). This pulp might suit as a feedstuff for dairy

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cows, and thereby improve the sustainable integrity of the concept of biorefining.

In previous experiments, whole-plant silage has been substituted with either silage of pulp from fractionated fresh forage or with silage pulp. The substitutions showed that milk production in dairy cows increased (Damborg et al., 2019), tended to decrease (Savonen et al., 2020), and decreased (Sousa et al., 2022). In Damborg et al. (2019) and Sousa et al. (2022), DMI was not affected, when cows were fed pulp compared with the corresponding whole-plant silage. However, DMI was higher at a 25:75 ratio of silage pulp to chopped silage, compared with a 0:100 and 50:50 ratio of silage pulp to chopped silage (Savonen et al., 2020). The effects on DMI and milk production were probably attributed to increased NDF digestibility and protein value (Damborg et al., 2018, 2019), which might be further altered by the number of fractionations of the harvested plant material.

During fractionation, the plant is subjected to heavy physical maceration, with the aim of extracting as much soluble protein possible, while the plant material is reduced in size, partly disintegrated, and the surface area increased, which potentially could increase digestibility (Buxton and Redfearn, 1997). The DM yield is higher when forage is harvested at late compared with early developmental stage, but NDF concentration is higher and NDF digestibility is lower (Rinne et al., 1997) due to increased lignification and stem proportion. This implies that for forage harvested at a late compared with early developmental stage, the potential for increasing NDF digestibility may be higher from fractionation due to the disintegration of lignified tissues and stems.

The heavy maceration of plant particles during fractionation probably results in different sizes and structures of pulp particles relative to chopped whole-plant particles. Consequently, fractional rate of ruminal degradation and passage of NDF out of the rumen may be affected (Buxton and Redfearn, 1997), such that NDF digestibility of the treated plant material, caused by fractionation in the current study, is different compared with that of the whole plant, as seen for other types of physical processing (Koegel et al., 1992; Hansen et al., 2021). Improving the extraction of protein by increasing the number of fractionations may further improve NDF digestibility in cows, but has not been investigated yet. Moreover, fractionation of grass-clover has resulted in contradicting effects on OM digestibility (**OMD**) of pulp silage and whole-plant silage depending on whether a conventional laboratory method or in vivo experiment was used for determination (Damborg et al., 2019). Therefore, actual knowledge on effects of fractionation on in vivo determined ruminal kinetics of pulp compared with the whole plant is needed to relate effects on degradation to milk performance.

The proportion of soluble CP is lower in pulp compared with the whole plant (Damborg et al., 2018) and consequently, although some soluble CP can escape ruminal degradation (Reynal et al., 2007), the proportion of RUP might be higher in pulp, leaving a larger proportion of feed CP for potential small intestinal digestion. Hellwing et al. (2018) reported higher CH_4 yield (L/kg of DMI) in heifers fed only pulp silage compared with only silage of the whole plant. This is also expected for dairy cows, as the NDF concentration probably is higher in pulp compared with the whole plant (Boadi et al., 2004), but dairy cows are often fed concentrate alongside the silages, which might affect the outcome.

The objective of the current study was to investigate the effects of substituting whole-plant silage of chopped grass with pulp silage from grass fractionated once or twice at 2 developmental stages from the same field on nutrient digestion and production of CH_4 in dairy cows. We hypothesize that, compared with whole-plant grass silage, increasing the number of fractionations results in pulp silage having (1) increased apparent NDF digestibility and that the effect depends on developmental stage of grass at harvest, (2) reduced true ruminal CP digestibility and increased small intestinal digestion of AA, and (3) increased CH_4 yield.

MATERIALS AND METHODS

The experiment complied with the guidelines set out by the Danish Ministry of Environment and Food (2014) Law No. 474 (May 15, 2014) concerning animal experiments and care of animals used for scientific purposes.

Experimental Silages

A field of perennial ryegrass (*Lolium perenne* L., a mixture of the varieties Garbor, Bovini 1, Vsaqui, and Masai) was established in 2018 on a location near Tjele, Denmark (56.49° N 9.60° E). The grass was harvested in third regrowth at early (35 d of regrowth) and late (44 d of regrowth) developmental stage in 2019 on August 27 and September 4, respectively. Within each developmental stage, grass was either mowed (7 cm stubble height), wilted, and chopped (JF FCT 900, Kongskilde Industries A/S; 15 mm theoretical length of chopping) before being ensiled or harvested (GT 140, Future Grass technology Ltd.) whole at 7 cm stubble height and brought directly to the biorefinery demonstration platform in AU Foulum. The grass intended for chopped grass silage, was wilted for only 24 and 16 h at the early and late developmental stage, respectively,

to avoid loss of nutrients due to forecasted rain. The grass was fractionated into a solid pulp fraction and a liquid fraction using a twin screw press (P25, CirTech A/S). Half of the pulp produced during fractionation was ensiled in airtight plastic barrels (200 L; Jysk Emballage Rens) without addition of additives. The other half was rehydrated at a ratio of 2:1 (pulp:H_2O) and fractionated a second time in the same screw press before being ensiled in the same way as the pulp from the first fractionation. Within each developmental stage, 10 samples of grass, collected by grab-sampling during delivery of harvested material for the biorefinery, were pooled and divided into stems (leave sheath, stem, and flower) and leaves to determine the stem proportion on DM basis (60°C for 48 h in air-forced oven).

Experimental Design, Animals, and Housing

Six multiparous Holstein cows cannulated in the rumen (#1C, Bar Diamond Inc.), duodenum, and ileum (simple T-shaped; 25 mm diameter) were used in the current study. The cows were housed in a tiestall with rubber mattresses, sawdust as bedding material, and given free access to water. At the beginning of the experiment, the cows averaged (mean ± standard deviation): ECM yield, 30.7 ± 3.7 kg/d; DMI, 20.6 ± 2.6 kg/d; DIM, 176 ± 93 d; BW, 617 ± 41 kg. The 6 experimental silages were mixed into 6 treatments and were fed to the cows that were randomly assigned to the treatments in an incomplete 6 × 4 Latin square design with 4 periods and a 2 × 3 factorial arrangement of treatments; 2 developmental stages (early and late) and 3 levels of processing (chopped, fractionating once, and fractionating twice). Each experimental period lasted 21 d and constituted a period for adaptation, digesta sampling, and gas exchange measurement. The experimental periods started in a staggered order for the 6 cows because the capacity for measuring gas exchange was limited to 4 cows at a time (described below). Therefore, each reference given to a d within a period in the following sections refers to 4 cows, and the number given in brackets refers to the last 2 cows.

Composition of experimental silages are given in Table 1. The silages were fed as a TMR and consisted of 650, 68.2, 264, 13.6, and 4.6 g/kg on DM basis of experimental silage, soybean meal [75 g ash, 91 g ash-free neutral detergent fiber (**aNDFom**), and 501 g CP/kg of DM, respectively, 18 g indigestible aNDFom (**iNDF**)/kg of aNDFom and 148 g soluble N/kg of N], rolled wheat (15 g ash, 102 g aNDFom and 114 g CP/kg of DM, respectively, 180 g iNDF/kg of aNDFom and 264 g soluble N/kg of N), mineral mix (VM2 grøn, Vilofoss; Ca, 160 g/kg; P, 50 g/kg; Mg, 65 g/kg; Na, 90 g/kg; S, 2 g/kg; Mn, 4,000 mg/kg; Zn, 4,500 mg/kg; Cu, 1,500 mg/kg; Co, 25 mg/kg; I, 225 mg/kg; Se, 50 mg/kg; vitamin A, 600 IU/g; vitamin D_3, 190 IU/g; vitamin E, 4,000 IU/kg), and monocalcium phosphate, respectively. The 6 types of TMR were based on silage of either chopped grass (**GS**), pulp from one fractionation (**1×P**), or pulp from 2 fractionations (**2×P**), harvested either at early or late developmental stage. In combination, the 6 TMR treatments were denoted **EGS**, **E1×P**, **E2×P**, **LGS, L1×P**, and **L2×P** for GS, 1×P, and 2×P harvested at early developmental stage and GS, 1×P, and 2×P harvested at late developmental stage, respectively. The TMR were mixed in a Cormall auger feed-mixer (Cormall A/S) once weekly, vacuum-packed in plastic bags (130 μm thick; 12 kg/bag), and stored in a fridge at maximum 4°C until feeding. The cows were fed for ad libitum intake twice daily at 0715 and 1710 h; feed refusals were removed and weighted before the evening feeding, and the amount of new feed offered were adjusted aiming at 10% refusals. The cows were milked twice daily at 0600 and 1610 h. Two external markers were used to determine the flow and digestibility of nutrients. The markers were added directly in the rumen in separate degradable filter paper bags [10 g chromium(III) oxide and 13 g titanium(IV) dioxide] during each milking.

Sampling and Recording

Samples of silages, soybean meal, and wheat were collected upon mixing of the TMR on d 7 (5) and d 14 (12) within each period and stored at −20°C. After the experiment, thawed samples of silage were pooled within silage type and period (n = 4) and thawed samples of each concentrate were pooled within period 1 and 2 and period 3 and 4 (n = 2). Extracts of silages were prepared for analysis of fermentation products and buffer capacity by homogenizing 100 g of the pooled samples of silages in 1 L of water. After centrifuging the homogenate, pH was measured in the supernatant, which was stored in duplicate (with and without 25% meta-phosphoric acid) at −20°C until analysis. Pooled samples of silages and concentrates were stored at −20°C until chemical and in situ analysis.

Samples of TMR and TMR refusals were collected during 5 consecutive d starting on d 12 (10) and d 13 (11), respectively, to determine DM concentration (60°C for 48 h), and subsequently to calculate the average DMI per cow per period. Milk yield and composition was determined on the same d as DMI and also averaged per cow per period.

To cover the diurnal variation, 12 samples of duodenal digesta (400 mL), ileal digesta (200 mL), and feces

Table 1. Chemical composition (g/kg of DM unless otherwise stated) of experimental silages and extracts[1] (n = 4)

	Treatment							*P*-value[3]		
	Early			Late						
Item	Grass, chopped	Pulp, fractionated once	Pulp, fractionated twice	Grass, chopped	Pulp, fractionated once	Pulp, fractionated twice	SEM[2]	Dev	Pro	Dev × pro
DM, g/kg	266^{c}	272^{c}	390^{a}	200^{d}	296^{b}	377^{a}	3.2	<0.01	<0.01	<0.01
Ash	98.3^{a}	64.3^{c}	37.9^{e}	91.8^{b}	56.2^{d}	37.6^{e}	1.32	<0.01	<0.01	0.02
OM	902^{e}	936^{c}	962^{a}	908^{d}	944^{b}	962^{a}	1.3	<0.01	<0.01	0.02
aNDFom[4]	452^{d}	527^{c}	669^{a}	487^{d}	593^{b}	692^{a}	7.7	<0.01	<0.01	0.04
iNDF,[5] g/kg of aNDFom	83.9^{b}	95.3^{ab}	98.8^{ab}	109^{a}	102^{a}	99.7^{a}	3.37	<0.01	0.68	0.01
iNDF	37.9^{e}	50.2^{d}	66.0^{ab}	53.0^{cd}	60.2^{bc}	68.9^{a}	1.83	<0.01	<0.01	0.02
Hemicellulose	198^{c}	215^{c}	314^{a}	201^{c}	254^{b}	314^{a}	5.2	<0.01	<0.01	<0.01
ADF	254^{z}	312^{y}	355^{x}	286^{z}	339^{y}	378^{x}	3.0	<0.01	<0.01	0.41
Cellulose	242^{z}	297^{y}	337^{x}	268^{z}	319^{y}	358^{x}	3.4	<0.01	<0.01	0.80
ADL	12.1	15.5	17.7	18.1	20.3	19.8	1.63	0.01	0.09	0.49
CP	182	187	188	159	163	164	2.2	<0.01	0.05	0.97
NDIN	3.26^{cd}	4.08^{c}	10.4^{a}	2.61^{d}	3.95^{c}	6.86^{b}	0.210	<0.01	<0.01	<0.01
ADIN	1.49^{y}	1.51^{xy}	1.67^{x}	1.45^{y}	1.48^{xy}	1.55^{x}	0.053	0.13	0.02	0.64
Total AA	117^{b}	118^{b}	129^{a}	85.1^{d}	98.6^{c}	98.3^{c}	1.77	<0.01	<0.01	0.01
N, g/kg of total N										
Soluble N	594^{a}	535^{bc}	306^{d}	575^{ab}	512^{c}	346^{d}	10.7	0.94	<0.01	0.02
Ammonia N	36.3	40.2	39.4	43.8	39.4	41.4	8.24	0.68	1.00	0.88
AA-N	533^{b}	527^{b}	590^{a}	446^{c}	505^{b}	505^{b}	8.0	<0.01	<0.01	<0.01
NDIN	112de	136^{cd}	345^{a}	103^{e}	152^{c}	263^{b}	7.6	<0.01	<0.01	<0.01
ADIN	51.3	50.6	55.4	57.2	56.9	59.2	1.85	<0.01	0.08	0.72
OMD,[6] g/kg	789^{x}	777^{y}	754^{z}	753^{x}	743^{y}	734^{z}	3.27	<0.01	<0.01	0.06
pH	4.18^{a}	3.99^{c}	3.96^{c}	4.07^{b}	3.95^{c}	3.97^{c}	0.018	<0.01	<0.01	0.02
TFA[7]	31.9^{b}	29.1^{bc}	18.4^{d}	41.5^{a}	26.4^{c}	19.2^{d}	0.73	<0.01	<0.01	<0.01
Acetate	29.9^{b}	25.3^{c}	15.8^{d}	36.4^{a}	22.9^{c}	16.5^{d}	0.53	<0.01	<0.01	<0.01
Propionate	1.75^{b}	3.56^{ab}	2.42^{b}	4.84^{a}	3.33^{ab}	2.58^{b}	0.410	0.01	0.08	<0.01
Caproate	0.140^{ab}	0.185^{ab}	0.127^{ab}	0.243^{a}	0.0815^{b}	0.132^{ab}	0.0287	0.95	0.08	0.01
L-Lactate[8]	37.6^{b}	46.8^{a}	27.3^{c}	46.4^{a}	40.8^{b}	28.3^{c}	0.84	0.09	<0.01	<0.01
Glucose	1.52	1.62	1.31	0.957	1.11	1.06	0.2327	0.03	0.74	0.77
BC,[9] mEq/100 g of DM	26.2^{a}	20.7^{b}	16.7^{cd}	19.2^{bc}	19.5^{b}	16.2^{d}	0.54	<0.01	<0.01	<0.01

[a–e]Values within the same row with different superscripts differ due to interaction between developmental stage and processing ($P \leq 0.05$).

[x–z]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Extracts were also analyzed for butyrate, but it was not detected.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Ash-free neutral detergent fiber.

[5]Indigestible aNDFom.

[6]In vivo OM digestibility, calculated as 410 + 0.959 × in vitro OMD (Åkerlind et al., 2011).

[7]Total fermentation acids.

[8]L-Lactate constitutes about half of total lactate (Johansen et al., 2020).

[9]Buffer capacity (Playne and McDonald, 1966).

(300 mL) were collected during d 13 (11) to 17 (15) in each period [d 13 (11): 1000 h, 1800 h; d 14 (12): 0200 h, 1200 h, 2000 h; d 15 (13): 0400 h, 1400 h, 2200 h; d 16 (14): 0600 h, 1600 h, 2400 h; d 17 (15): 0800 h]. The samples were pooled and stored at −20°C before chemical analysis. On the same 12 time points, ruminal fluid was collected from the ventral ruminal sac through the ruminal cannula, using a plastic syringe mounted to a suction strainer. Immediately after sampling, ruminal fluid pH was measured and the samples were stored at −20°C before chemical analysis.

Gas exchange was measured for each cow during a 72-h period from d 17 to 20 (18 to 21) in individual 17-m^3 respiration chambers (Hellwing et al., 2012). Because only 4 chambers were available, the cows were staggered in a way that, within each period, 4 cows entered the chambers first and then the last 2 cows. During the period of gas exchange measurement, chambers were accessed twice daily to milk and feed the cows, and clean the stalls. Samples of TMR and TMR refusals were collected on the 3 d during the gas measurement to determine DM concentration (60°C for 48 h) and subsequently DMI in the period of gas exchange measurement for each cow per period. Milk yield was determined for the same 3 d and also averaged per cow per period for the period of gas exchange measurement.

The concentrations of CH_4, CO_2, O_2, and H_2 were measured every 12.5 min in all periods in air exhaust from each of the chambers and the background air (inlet air). The air flow was measured using an HFM-200 flow meter with a laminar flow element (Teledyne Hastings Instruments). The concentration of CH_4 was measured using an infrared sensor (VIA-510, Horiba instruments), CO_2 using an infrared sensor and O_2 with a paramagnetic sensor, both from Columbus Instruments International, and the concentration of H_2 using an electrochemical sensor (3HYT CiTiceL, Honeywell International Inc.). Gas exchange data were deleted from time points, where chamber doors were open. The 24-h gas exchange was calculated as accumulated gas over the total measuring period divided by the total measuring time in min multiplied by 1,440 min. Recovery tests were performed before the experiment, between periods, and after the experiment and used for individual chamber correction. In total, 57 CO_2 recovery tests with a recovery of 99.3 $\pm$ 1.4% and 25 CH_4 recovery tests with a recovery of 99.1 $\pm$ 1.6% were performed. A few H_2 recovery tests were performed together with CO_2 and CH_4 recovery tests and the recovery was close to 100%, but there was not enough observations to make a recovery factor. Recovery (disappearance) test for O_2 was not conducted. As the recoveries for CO_2 and CH_4 were close to 100%, it was assumed that the flow measured was close to 100%. All sensors were calibrated and adjusted before all measurements and no problems were observed during the experiment. Based the 2 above assumptions it was decided to correct the H_2 and O_2 with the average of the average CO_2 and CH_4 recoveries. Gas production is reported in L under standard conditions (0°C, 101.325 kPa).

To avoid carryover effects on measurements of gas exchange, ruminal evacuations were performed at 1145 h on d 15 for the 2 cows entering the respiration chambers last, and at 1145 h on d 21 for the 4 cows entering the respiration chambers first. The time point for ruminal evacuation was chosen to obtain samples and recordings representative for the diurnal variation in ruminal pools (Lund, 2002). All ruminal content was placed manually into a sieve basket, through which the liquid fraction of ruminal content was allowed to run into a tub, while the solid fraction remained in the sieve basket. After weighing and mixing, subsamples were taken from the solid and liquid fractions, and 3 composited samples (500 g) were made proportionally to the weight of each fraction, making representative samples for the whole ruminal content. The composited samples were either used for DM determination (60°C for 48 h) or stored at −20°C until chemical and in situ analysis. At 1000 h on d 17 (15), 3.0 L of ruminal fluid was collected for harvest of microbes by differential centrifugation as described by Johansen et al. (2017). Milk samples were collected at 2 occasions; during 6 consecutive milkings starting on d 13 (11) and during the last 4 consecutive milkings, in the period where gas exchange was measured.

Chemical Analyses

Pooled samples of silages, concentrates, microbial pellet, duodenal digesta, ileal digesta, feces, and ruminal contents were freeze-dried and milled through a 1 mm screen (ZM 200 mill, Retsch GmbH) before chemical analysis. In all samples, ash was determined by combustion at 525°C for 6 h. Nitrogen was analyzed following the Dumas principle (Hansen, 1989) using a Vario Max CN (Elementar Analysesysteme GmbH) and CP was calculated as N $\times$ 6.25. Soluble N was determined in silages and concentrates by extraction in a 39°C borate-phosphate buffer at pH 6.75 for 1 h (Åkerlind et al., 2011). All samples, except microbial pellet, were analyzed sequentially for aNDFom, ADF, and ADL following the ANKOM procedure using heat-stable α-amylase and sodium sulphite (Mertens, 2002) and corrected for residual ash after the ADL procedure. The concentration of hemicellulose was calculated as the difference between aNDFom and ADF, and cellulose was calculated as the difference between ADF and ADL. After use of a Fibertec M6 system (FOSS Analytical) for the NDF and ADF procedure, silage sample residues were hydrolyzed using sulfuric acid, and NDIN and ADIN, respectively, were determined using a Kjeltec 2400 (FOSS Analytical).

Duodenal digesta, ileal digesta, and feces were analyzed for chromium(III) oxide by spectrophotometry after oxidation with sodium peroxide chromate (Schürch et al., 1950). The same samples were analyzed for titanium(IV) dioxide as described by Myers et al. (2004) with the modification that 15 instead of 10 mL of 30% hydrogen peroxide were added and that 5 additional drops of hydrogen peroxide were added before measuring the absorbance. To determine microbial synthesis in the rumen, microbial pellet and duodenal digesta were analyzed for purines by spectrophotometry as described by Zinn and Owens (1986) but with the modification that the washing procedure was done using a solution of sulfuric acid and silver nitrate as described by Thode (1999). The AA were analyzed in silages, concentrates, microbial pellet, duodenal digesta, and ileal digesta using UPLC (Dahl-Lassen et al., 2018). Here, sulfur-containg AA were oxidized with performic acid and hydrolyzed with hydrochloric acid (110°C for 24 h), and the total amount of AA was calculated as the sum of Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, and Val.

The silage in vitro OMD was determined following the procedure by Tilley and Terry (1963), where samples of silage were incubated in ruminal fluid for 48 h and subsequently in a hydrochloric acid and pepsin solution for 48 h. The in vivo OMD was then calculated as 410 + 0.959 × in vitro OM digestibility as described in Åkerlind et al. (2011).

Indigestible aNDFom was determined in silages, concentrates, ruminal contents, and feces by incubating Dacron bags (12 µm pore size, Saatitech S.p.A.) with 2 g freeze-dried and milled samples (1.5 mm; Pulverisette 15, Fritsch GmbH) in 3 nonlactating cows (3 replicates; 1 bag per cow per pooled sample) for 288 h as described by Krämer et al. (2012). Residues remaining in bags after incubation were quantitatively transferred to filter crucibles and analyzed for aNDFom using the Fibertech M6 system (Foss Analytical) and referred to as iNDF.

In samples of ruminal fluid and silage extracts containing meta-phosphoric acid, individual VFA was analyzed using GC (Kristensen et al., 1996), ammonia N was analyzed using a Cobas Mira autoanalyzer (Triolab A/S), and L-lactate and glucose were analyzed using an YSI 2900D (YSI Inc.) following the immobilized oxidase electrode technique (Mason, 1983). The sum of individual VFA and L-lactate was referred to as fermentation acids. Buffer capacity was measured on silage extracts without meta-phosphoric acid as the mEq alkaline required to raise pH from 4 to 6 per 100 g of DM (Playne and McDonald, 1966) using a Titrator Excellence T7 (Mettler Toledo).

Milk samples were analyzed for crude protein, fat, and lactose monohydrate using a Milkoscan 4000 analyzer (Foss Analytical) at Eurofins Steins.

Calculations

The DM flow in duodenum and ileum and the DM output in feces were calculated using each marker separately, averaged, and then used for calculating nutrient flow and output. Assuming that purines in duodenal digesta was only of microbial origin, duodenal flow of microbial DM (kg/d) was calculated as: duodenal flow of purines (kg/d) / purine concentration in microbial pellet (g/kg DM) × 1,000. Nutrient flows and digestion were related to DMI for feed evaluation purposes. Using duodenal flow of aNDFom have previously yielded unreliable feed to duodenum ruminal digestibility of aNDFom (Brask et al., 2013), and therefore, ileal flow of aNDFom was used to also calculate feed to ileum apparent ruminal digestibility. The true ruminal digestibility of DM, OM, and CP was calculated by correcting for duodenal flow of microbial DM, OM, and CP, respectively. Efficiency of the microbial CP synthesis was expressed as the duodenal flow of microbial CP per kg of aNDFom (feed to ileum) or OM truly degraded in the rumen. Based on Robinson et al. (1987), ruminal fractional rate of degradation ($\mathbf{k_d}$; %/h) of digestible aNDFom (**DNDF**; calculated as aNDFom minus iNDF) was calculated as: [DNDF intake (kg/d) – fecal output of DNDF (kg/d)/24]/ruminal pool of DNDF (kg) × 100. The ruminal fractional rate of passage ($\mathbf{k_p}$; %/h) of iNDF was calculated as: [Fecal flow of iNDF (kg/d)/24]/ruminal pool of iNDF (kg) × 100.

Yield weighted averages of fat, CP, and lactose were used to calculate the ECM (3.14 MJ/kg) yield across d within the digesta sampling period and within the period of gas exchange measurement, where milk yield was measured. The ECM yield was calculated as described by Sjaunja et al. (1991): ECM yield (kg/d) = milk yield (kg) × [(38.3 × fat (g/kg) + 24.2 × crude protein (g/kg) + 15.71 × lactose (g/kg) + 20.7) / 3,140], where lactose is lactose monohydrate. The N use efficiency was calculated as: [milk crude protein (kg/d) / 6.38] / N intake (kg/d) × 100. The CH_4 production was expressed in relation to the DMI and ECM yield determined during the period of gas exchange measurement, and referred to as CH_4 yield and CH_4 intensity, respectively.

Statistical Analysis

The statistical analyses were conducted using R 4.0.4 (R Core team, 2021) and the effects of developmental stage and processing were analyzed using the mixed linear model in Equation 1 with the 'lmer' function from the 'lme4' package (Bates et al., 2015):

$$Y_{dptc} = \mu + \alpha_d + \beta_p + (\alpha\beta)_{dp} + \tau_t + A_c + \varepsilon_{dptc} \quad [1]$$

For the model in Equation 1, Y_{dptc} is the dependent response variable (n = 4), μ is the overall mean, α_d is the fixed effect of developmental stage (d = E, L), β_p is the fixed effect of processing (p = GS, 1×P, 2×P), $(\alpha\beta)_{dp}$ is the interaction between developmental stage and processing termed treatment (dp = EGS, E1×P, E2×P, LGS, L1×P, L2×P), τ_t is the fixed effect of period (t = 1, …, 4), and A_c is the random effect of cow (c = 1, …, 6). The random effect of cow A_c and the residual error ε_{dptc} were assumed to be normal distributed with zero mean and variance σ^2_A and σ^2_ε, respectively.

When analyzing the chemical composition of silages (Table 1), the model in Equation 1 was also used, except that the random effect of cow A_c was excluded. Variables for ruminal fluid pH, the concentrations of

ammonia N and glucose in ruminal fluid, and ruminal fluid fermentation acid proportions were analyzed using the model in Equation 1 and including the fixed effect of sampling time, the 3-way interaction of developmental stage, processing, and sampling time, and sampling time within cow and period as repeated measurement. Repeated measures were handled with a compound symmetry covariance structure chosen based on AIC. However, the 3-way interaction was not significant ($P > 0.05$) for any of the response variables. The response variables were therefore averaged per cow per period and tested using the model in Equation 1. The experimental design resulted in 4 observations per treatment unless otherwise stated in the tables. However, data on digesta from ileum from one cow receiving EGS, E1×P, E2×P, and LGS were not available due to cannula problems.

The 'emmeans' package was used to obtain least squares means (LSM) for each treatment and highest standard error of the mean, which are given in the tables. If the effect of the interaction between developmental stage and processing was significant, the differences between LSM were evaluated comparing a family of 6 (EGS, E1×P, E2×P, LGS, L1×P, L2×P) with adjustment of multiple testing using the Tukey's procedure. If the interaction between developmental stage and processing was not significant and the effect of processing was significant, the differences between LSM were evaluated comparing a family of 3 (GS, 1×P, 2×P) with adjustment of multiple testing using the Tukey's procedure. Statistical significance was regarded when $P \leq 0.05$ and as tendencies when $0.05 < P \leq 0.10$.

RESULTS

Silages

The stem proportion averaged 75 and 115 g/kg on DM basis (not shown) for grass harvested at the early and late developmental stage, respectively.

The effect of processing on DM concentration depended on the developmental stage ($P < 0.01$) because silage of chopped grass and pulp from grass fractionated once were similar at early developmental stage but different ($P < 0.01$) at late developmental stage (Table 1). The effect on ash concentration of processing interacted with developmental stage ($P = 0.02$), but showed that within each developmental stage, ash concentration was highest for silage of chopped grass, followed by pulp silage of grass fractionated once, which was higher than pulp silage of grass fractionated twice. In contrast, pulp silage of grass fractionated twice compared with silage of chopped grass had 217 and 205 g/kg of DM higher ($P < 0.01$ and $P < 0.01$, respectively) aNDFom concentration, when grass was harvested at early and late developmental stage, respectively. Compared with pulp silage of grass fractionated once and twice, silage of chopped grass had 12 and 27 g/kg higher ($P = 0.01$ and $P < 0.01$, respectively) in vitro OMD, and compared with pulp silage fractionated twice, pulp silage of grass fractionated once had 16 g/kg higher ($P < 0.01$) in vitro OMD.

The CP concentration was lower (162 vs. 186 g CP/kg of DM; $P < 0.01$) in silages of grass harvested at late compared with early developmental stage. The CP concentration was also affected by processing ($P = 0.05$), but evaluation by multiple comparisons showed no significant difference between LSM (GS, 1×P, and 2×P). The effect of processing on both NDIN concentration (g/kg of DM) and proportion (g/kg of total N) depended on the effect of developmental stage ($P < 0.01$ and $P < 0.01$, respectively), showing highest NDIN concentration and proportion for pulp silage of grass fractionated twice within each developmental stage. In contrast, processing affected ($P = 0.02$) ADIN concentration, which was lower in silage of chopped grass compared with pulp of grass fractionated twice.

An interaction ($P = 0.02$) between developmental stage and processing showed that silage pH was highest for silage of chopped grass harvested at early developmental stage and lowest for all pulp silages. Within each developmental stage, buffer capacity was lowest ($P < 0.05$ for all comparisons) for pulp of grass fractionated twice, whereas buffer capacity in silage of chopped grass was higher ($P < 0.01$) than pulp silage of grass fractionated once harvested at early but not late developmental stage.

Feed Intake, Duodenal Flow, and Weight Changes

Compared with GS, 2×P had 2.6 and 0.4 kg lower ($P < 0.01$ and $P < 0.01$, respectively) daily intake of DM and CP, respectively, whereas aNDFom intake was 1.2 kg higher ($P < 0.01$; Table 2). Moreover, aNDFom intake was 0.3 kg higher ($P = 0.05$) and CP intake was 0.3 kg lower ($P < 0.01$), when grass was harvested at late compared with early developmental stage. The duodenal flow of OM was not affected by developmental stage or processing (Table 3). Duodenal flow (g/d) of total AA was not affected by processing, whereas duodenal flows (g/d) of microbial CP and AA were lower ($P = 0.01$ and $P = 0.01$, respectively) for 2×P compared with GS. The effect of processing on duodenal flow (g/kg of DMI) of AA of feed and endogenous origin depended on developmental stage ($P < 0.01$), showing that E2×P was higher ($P < 0.01$ and $P <$

Table 2. Feed intake (kg/d unless otherwise stated) in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
DM	17.8^{x}	16.3^{xy}	15.1^{y}	17.2^{x}	16.3^{xy}	14.7^{y}	0.67	0.45	<0.01	0.87
OM	16.2^{x}	15.2^{xy}	14.3^{y}	15.7^{x}	15.2^{xy}	14.0^{y}	0.62	0.52	0.01	0.85
CP	3.25^{x}	3.02^{xy}	2.79^{y}	2.86^{x}	2.75^{xy}	2.50^{y}	0.123	<0.01	0.01	0.79
aNDFom[4]	5.86^{z}	6.09^{y}	7.00^{x}	5.97^{z}	6.85^{y}	7.15^{x}	0.238	0.05	<0.01	0.20
Hemicellulose	2.72^{bc}	2.63^{c}	3.40^{a}	2.63^{c}	3.09^{ab}	3.37^{a}	0.107	0.12	<0.01	0.01
ADF	3.14^{y}	3.46^{x}	3.60^{x}	3.35^{y}	3.76^{x}	3.78^{x}	0.134	0.02	<0.01	0.85
Cellulose	2.97^{y}	3.27^{x}	3.39^{x}	3.11^{y}	3.52^{x}	3.56^{x}	0.134	0.06	<0.01	0.87
ADL	0.170	0.198	0.203	0.238	0.245	0.221	0.0166	0.01	0.55	0.33
iNDF[5]	0.530^{y}	0.611^{xy}	0.721^{x}	0.679^{y}	0.720^{xy}	0.734^{x}	0.0352	<0.01	0.01	0.12
Total AA-N	0.305	0.282	0.278	0.247	0.253	0.229	0.0108	<0.01	0.08	0.27
Total AA	2.28^{x}	2.10^{xy}	2.03^{y}	1.84^{x}	1.89^{xy}	1.70^{y}	0.081	<0.01	0.04	0.28
DM intake, % of BW	3.00^{x}	2.67^{y}	2.52^{y}	2.86^{x}	2.66^{y}	2.45^{y}	0.140	0.38	<0.01	0.82
aNDFom intake, % of BW	0.986^{y}	1.00^{y}	1.17^{x}	0.996^{y}	1.12^{y}	1.19^{x}	0.0531	0.13	<0.01	0.29

[a–c]Values within the same row with different superscripts differ due to interaction between developmental stage and processing ($P \leq 0.05$).

[x–z]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Ash-free neutral detergent fiber.

[5]Indigestible aNDFom.

0.01, respectively) than both EGS and E1×P, whereas L2×P was only higher ($P = 0.05$) than LGS and not L1×P. The duodenal flow (g/kg of DMI) of microbial AA was higher ($P = 0.04$) for GS compared with 1×P, resulting in E2×P having higher duodenal flow (g/kg of DMI) of total AA compared with all other treatments. Developmental stage and processing had no effect on daily BW change, and from the beginning to the end of the experiment, total BW change averaged −6.8 kg across treatments (data not shown).

Digestibility, Ruminal Kinetics, and Ruminal Fluid

The effect of processing on apparent ruminal digestibility of aNDFom and DNDF (feed to ileum for both) depended on developmental stage ($P = 0.04$ and $P = 0.04$, respectively) because treatments within early developmental stage did not differ, whereas a 104 and 99 g/kg higher ($P = 0.01$ and $P = 0.01$, respectively) apparent ruminal digestibility of aNDFom and DNDF (feed to ileum for both), respectively, were found for L2×P compared with LGS (Table 3). Apparent feed to duodenum ruminal NDF digestibility was affected by processing such that 2×P was 51 g/kg higher ($P = 0.05$) compared with GS, and no interaction between developmental stage and processing was present for this variable. The effect of processing on true ruminal digestibility of CP interacted with developmental stage ($P = 0.01$) showing that treatments within late developmental stage did not differ, whereas E2×P was 157 g/kg lower ($P < 0.01$) compared with EGS. Apparent small intestinal digestibility of CP and AA was not affected by processing. However, processing affected ($P = 0.01$) the AA digested in the small intestine (g/kg of DMI), showing that 2×P was 14 g/kg of DMI higher ($P = 0.01$) than GS, and that 1×P was not different from GS and 2×P (Table 4). The effect of processing on the efficiency of the microbial CP synthesis depended on developmental stage ($P = 0.04$), when it was related to aNDFom digested in the rumen (feed to ileum) but not OM. The reason was that the efficiency of the microbial CP synthesis for E1×P was higher ($P = 0.04$) than E2×P within early developmental stage, whereas at late developmental stage, L1×P was lower than LGS ($P < 0.01$) but not different from L2×P.

The total content of fresh material in the rumen was 17 kg higher ($P < 0.01$) for 2×P compared with GS, whereas 1×P did not differ from GS or 2×P (Table 5). In addition, ruminal pools of DM, OM, and aNDFom were affected by processing ($P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively), all variables being highest for 2×P, followed by 1×P, and lowest for GS. The ruminal pool size of iNDF was 0.47 and 0.36 kg

Table 3. Duodenal flow and nutrient digestibility in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4 unless otherwise stated)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
Duodenal flow (g/d)										
DM, kg/d	12.1	11.2	10.8	12.0	10.9	9.8	0.75	0.40	0.07	0.79
OM, kg/d	8.92	8.26	8.26	8.65	8.21	7.39	0.601	0.37	0.24	0.72
Total CP, kg/d	3.64	3.37	3.45	3.37	3.05	2.85	0.213	0.02	0.15	0.63
Total AA	2,297	2,100	2,238	2,085	1,911	1,787	141.4	0.02	0.26	0.51
Microbial CP	1,520^{x}	1,373^{xy}	1,286^{y}	1,503^{x}	1,161^{xy}	1,065^{y}	99.1	0.08	0.01	0.52
Microbial AA	987^{x}	871^{xy}	816^{y}	966^{x}	756^{xy}	696^{y}	63.5	0.12	0.01	0.68
Feed + endogenous AA	1,288	1,237	1,436	1,134	1,134	1,098	93.7	<0.01	0.44	0.17
Duodenal flow (g/kg DMI)										
DM	673	684	729	698	668	671	33.8	0.38	0.57	0.23
OM	493	505	556	504	502	504	27.2	0.34	0.23	0.23
Total CP	203	206	231	196	187	194	8.9	<0.01	0.05	0.08
Total AA	128^{b}	128^{b}	150^{a}	121^{b}	117^{b}	122^{b}	6.1	<0.01	0.02	0.05
Microbial CP	86.1	83.4	86.3	87.4	71.0	71.1	4.01	0.02	0.07	0.12
Microbial AA	55.8^{x}	53.0^{y}	54.7^{xy}	56.1^{x}	46.2^{y}	46.5^{xy}	2.39	0.02	0.04	0.20
Feed + endogenous AA	71.0^{bc}	76.1^{b}	96.0^{a}	66.4^{c}	69.6^{bc}	74.9^{b}	4.92	<0.01	<0.01	<0.01
Apparent ruminal digestibility, g/kg										
DM	327	316	271	302	332	329	33.8	0.38	0.57	0.23
OM	457	458	413	448	464	468	29.4	0.29	0.60	0.28
CP	−111	−114	−248	−177	−103	−140	52.4	0.57	0.11	0.10
aNDFom (feed to duodenum)[4,5]	766^{y}	787^{xy}	784^{x}	711^{y}	774^{xy}	794^{x}	19.2	0.21	0.04	0.25
aNDFom (feed to ileum)[6,7]	737^{ab}	721^{ab}	740^{ab}	662^{b}	717^{ab}	766^{a}	18.6	0.27	0.02	0.04
DNDF[6,7,8]	805^{ab}	793^{ab}	810^{ab}	743^{b}	803^{ab}	843^{a}	18.9	0.80	0.02	0.04
Hemicellulose[6,7]	666^{a}	629^{ab}	701^{a}	552^{b}	648^{ab}	725^{a}	25.2	0.26	<0.01	0.02
Cellulose[6,7]	846	834	821	796	821	844	13.2	0.27	0.49	0.04
True ruminal digestibility, g/kg										
DM	480	462	420	456	460	457	29.5	0.77	0.19	0.18
OM	598	588	546	588	579	579	25.3	0.67	0.12	0.22
CP	369^{a}	334^{ab}	211^{c}	335^{ab}	325^{ab}	284^{b}	38.9	0.40	<0.01	0.01
Apparent small intestinal digestibility, g/kg										
OM[7]	587^{ab}	602^{a}	548^{ab}	533^{b}	542^{ab}	552^{ab}	27.9	0.01	0.41	0.05
CP[7]	742	754	734	722	725	736	9.5	0.01	0.57	0.08
AA[7]	768	788	788	768	778	786	11.1	0.55	0.17	0.88
Apparent total-tract digestibility, g/kg										
DM	776^{a}	778^{a}	739^{b}	757^{ab}	750^{ab}	761^{ab}	8.8	0.20	0.11	0.02
OM	790^{ab}	797^{a}	759^{b}	776^{ab}	769^{ab}	779^{ab}	8.1	0.20	0.09	0.01
CP	722^{ab}	738^{a}	694^{b}	702^{ab}	706^{ab}	721^{ab}	8.8	0.26	0.25	0.01
aNDFom	760^{ab}	770^{a}	765^{ab}	715^{b}	740^{ab}	784^{a}	12.9	0.06	0.02	0.04
DNDF[8]	831^{y}	846^{xy}	840^{x}	801^{y}	829^{xy}	862^{y}	11.7	0.33	0.02	0.07
Hemicellulose	713^{ab}	726^{a}	753^{a}	649^{b}	708^{ab}	769^{a}	16.1	0.08	<0.01	0.05
Cellulose	850	854	824	815	822	840	10.6	0.04	0.77	0.03

[a–c]Values within the same row with different superscripts differ due to interaction between developmental stage and processing ($P \leq 0.05$).

[x,y]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Ash-free neutral detergent fiber.

[5]Apparent feed to duodenum ruminal digestibility.

[6]Apparent feed to ileum ruminal digestibility.

[7]Number of observations = 3 for treatments: Early (GS, 1×P, and 2×P); Late (GS).

[8]Digestible aNDFom. For intake, DNDF = feed aNDFom – feed indigestible aNDFom. For feed to ileum ruminal digestibility, ileum DNDF = ileum aNDFom – feces indigestible aNDFom. For apparent total-tract digestibility, feces DNDF = feces aNDFom – feces indigestible aNDFom.

Table 4. Nutrient digestion and efficiency of microbial protein synthesis in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4 unless otherwise stated)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
Digested in the rumen, kg/d										
aNDFom[4,5,6]	4.30^{z}	4.61^{y}	5.28^{x}	3.95^{z}	4.94^{y}	5.52^{x}	0.166	0.40	<0.01	0.11
DNDF[5,6,7]	4.31^{z}	4.53^{y}	5.17^{x}	3.92^{z}	4.96^{y}	5.46^{x}	0.189	0.29	<0.01	0.08
Digested in the small intestine										
AA,[6] g/d	1,731	1,786	1,814	1,499	1,481	1,423	121.3	<0.01	0.94	0.61
AA,[6] g/kg of DMI	95.7^{y}	103^{xy}	118^{x}	88.0^{y}	89.5^{xy}	94.7^{x}	6.04	<0.01	0.01	0.15
Efficiency of microbial CP synthesis[8]										
g CP/kg of OM	163	153	164	162	134	133	11.7	0.07	0.20	0.39
g CP/kg of aNDFom[6]	350^{a}	327^{a}	247^{b}	353^{a}	236^{b}	195^{b}	16.3	<0.01	<0.01	0.04

[a,b]Values within the same row with different superscripts differ due to interaction between developmental stage and processing ($P \leq 0.05$).

[x–z]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Ash-free neutral detergent fiber.

[5]Apparent feed to ileum ruminal digestibility.

[6]Number of observations = 3 for treatments: Early (GS, 1×P, and 2×P); Late (GS).

[7]Digestible aNDFom. For intake, DNDF = feed aNDFom – feed indigestible aNDFom. For ileum flow, ileum DNDF = ileum aNDFom – feces indigestible aNDFom.

[8]Duodenal flow of microbial CP per kg of OM truly digested in the rumen and aNDFom apparently digested in the rumen (feed to ileum ruminal digestibility).

higher ($P < 0.01$ and $P < 0.01$, respectively) for 2×P and 1×P compared with GS. Developmental stage and processing had no effect on k_d of DNDF or k_p of iNDF determined from ruminal evacuations. Ruminal pH and concentration of total fermentation acids in ruminal fluid were not affected by developmental stage or processing (Table 6). However, the proportion of acetate was higher in 2×P compared with GS and 1×P ($P < 0.01$ and $P < 0.01$, respectively) and lower in GS compared with 1×P ($P = 0.04$). Moreover, the proportion of butyrate was higher ($P < 0.01$ and $P < 0.01$, respectively) in GS compared with 1×P and 2×P. An effect ($P = 0.01$) of processing caused 2×P to have lower ($P < 0.01$) concentration of ammonia N in ruminal liquid compared with 1×P.

Milk and Production of CH_4

The ECM yield averaged 23.9 kg/d across treatments, and was not affected by developmental stage or processing (Table 7). The N use efficiency (milk N as % of N intake) was also not affected by developmental stage or processing, whereas feed efficiency was 0.14 kg ECM/kg of DMI higher ($P = 0.04$) for 2×P compared with GS, and 1×P was not different from GS and 2×P.

Compared with late developmental stage, early developmental stage had 3.2 L/kg of DMI and 2.4 L/kg of ECM lower ($P < 0.01$ and $P < 0.01$, respectively) CH_4 yield and intensity, respectively (Table 8). However, processing had no effect on either CH_4 yield or intensity. Processing affected hydrogen production such that GS was higher ($P < 0.01$ and $P < 0.01$, respectively) compared with 1×P and 2×P.

DISCUSSION

This study compared traditionally chopped grass silage with pulp silage from a biorefinery on fiber digestibility, feed protein value, and production of CH_4 using lactating dairy cows. The grass for traditional ensiling was cut and the grass fractionated in the biorefinery was harvested on the same d in the same field. It is one of few studies, which has investigated the value of pulp silage from green forages in vivo for lactating dairy cows, and it is the first in vivo study to enlighten the passage kinetics and digestion of fiber in the rumen and to quantify the protein value and the production of CH_4 from dairy cows fed pulp silage. Overall, we found that the processing interacted with the developmental stage of the grass at harvest. Apparent aNDFom digestibility was higher for pulp silage of grass fractionated

Table 5. Ruminal contents and ruminal kinetics of fiber in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
Total content, kg	86.4^{y}	96.1^{xy}	105^{x}	91.6^{y}	99.9^{xy}	108^{x}	6.72	0.22	<0.01	0.95
Free fluid, kg	27.3	26.9	25.2	24.6	30.1	25.3	2.36	0.89	0.20	0.30
Free fluid, % of total	32.0^{x}	28.0^{x}	24.3^{y}	27.0^{x}	29.8^{x}	23.4^{y}	1.96	0.25	<0.01	0.10
DM, g/kg	113^{y}	120^{xy}	123^{x}	115^{y}	117^{xy}	125^{x}	2.9	0.98	0.01	0.56
Pools, kg										
DM	9.79^{z}	11.5^{y}	12.8^{x}	10.5^{z}	11.7^{y}	13.3^{x}	0.74	0.18	<0.01	0.84
OM	8.82^{z}	10.4^{y}	11.6^{x}	9.47^{z}	10.7^{y}	12.2^{x}	0.674	0.14	<0.01	0.85
aNDFom[4]	4.93^{z}	5.99^{y}	6.92^{x}	5.49^{z}	6.16^{y}	7.26^{x}	0.482	0.11	<0.01	0.74
iNDF[5]	1.23^{y}	1.79^{x}	1.81^{x}	1.70^{y}	1.85^{x}	2.05^{x}	0.122	<0.01	<0.01	0.12
DNDF[6]	3.69^{y}	4.21^{y}	5.10^{x}	3.80^{y}	4.31^{y}	5.21^{x}	0.389	0.54	<0.01	1.00
Rates, %/h										
K_d DNDF[7]	5.42	4.76	4.46	4.79	4.97	4.43	0.537	0.61	0.21	0.49
K_p iNDF[8]	1.70	1.33	1.52	1.62	1.64	1.36	0.133	0.78	0.16	0.13

[x–z]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Ash-free neutral detergent fiber.

[5]Indigestible aNDFom.

[6]Digestible aNDFom = aNDFom – iNDF.

[7]Fractional rate of digestion of DNDF in the rumen.

[8]Fractional rate of passage of iNDF out of the rumen.

Table 6. Ruminal fluid pH and composition in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
pH	6.38	6.32	6.44	6.40	6.31	6.34	0.045	0.39	0.20	0.33
TFA,[4] mmol/L	129	134	125	129	138	132	4.6	0.34	0.22	0.77
TFA proportions, mol per 100 mol of total TFA										
L-Lactate[5]	0.27	0.23	0.10	0.22	0.34	0.15	0.075	0.59	0.12	0.59
Acetate	61.5^{z}	63.4^{y}	66.5^{x}	62.8^{z}	63.9^{y}	67.0^{x}	0.61	0.11	<0.01	0.69
Propionate	20.4	20.8	20.3	19.3	20.0	18.6	0.75	0.04	0.36	0.79
Isobutyrate	1.03^{xy}	1.05^{x}	0.937^{y}	0.945^{xy}	0.976^{x}	0.908^{y}	0.0421	0.01	0.01	0.47
Butyrate	12.7^{x}	10.5^{y}	8.86^{y}	12.6^{x}	11.1^{y}	10.1^{y}	0.603	0.16	<0.01	0.44
Isovalerate	2.06	2.19	1.91	2.07	2.10	1.95	0.243	0.90	0.28	0.86
Valerate	1.69^{a}	1.35^{b}	1.09^{cd}	1.41^{b}	1.25^{bc}	1.05^{d}	0.068	<0.01	<0.01	0.01
Caproate	0.40^{b}	0.39^{b}	0.23^{c}	0.56^{a}	0.40^{b}	0.30^{bc}	0.044	<0.01	<0.01	0.04
Acetate:propionate	3.04^{xy}	3.04^{y}	3.31^{x}	3.27^{xy}	3.22^{y}	3.63^{x}	0.145	0.03	0.03	0.83
Ammonia N, mmol/L	7.95^{xy}	9.63^{x}	5.33^{y}	6.68^{xy}	8.77^{x}	6.72^{y}	0.941	0.70	0.01	0.22
Glucose, mmol/L	0.97^{x}	0.67^{xy}	0.49^{y}	0.66^{x}	0.53^{xy}	0.51^{y}	0.108	0.11	0.02	0.29

[a–d]Values within the same row with different superscripts differ due to interaction between developmental stage and processing ($P \leq 0.05$).

[x–z]Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]Total fermentation acids.

[5]L-Lactate constitutes about half of total lactate (Johansen et al., 2020).

Table 7. Milk production in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
Yield										
Milk, kg/d	25.4	25.0	24.1	24.3	24.4	21.0	1.97	0.23	0.32	0.69
ECM,[4] kg/d	25.1	24.2	24.2	24.3	24.3	21.4	1.78	0.34	0.41	0.57
Fat, g/d	1,026	970	982	1,000	1,013	900	70.5	0.62	0.40	0.49
CP, g/d	857	838	839	819	797	717	66.6	0.17	0.58	0.70
Lactose, g/d	1,164	1,138	1,117	1,118	1,136	958	99.2	0.29	0.36	0.57
Composition, %										
Fat	4.07	3.95	4.10	4.17	4.15	4.29	0.192	0.07	0.39	0.87
CP	3.43	3.39	3.47	3.37	3.29	3.43	0.141	0.06	0.08	0.80
Lactose	4.56	4.58	4.62	4.58	4.64	4.56	0.110	0.66	0.35	0.16
NUE,[5] %	25.5	27.2	29.2	28.1	28.4	28.0	1.47	0.39	0.37	0.31
Kg of ECM/kg of DMI	1.38^{y}	1.48^{xy}	1.60^{x}	1.41^{y}	1.51^{xy}	1.46^{x}	0.076	0.48	0.04	0.16

^{x,y}Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]ECM calculated as described by Sjaunja et al. (1991).

[5]Nitrogen use efficiency = milk N as % of N intake.

twice compared with silage of chopped grass, but only for grass harvested at late and not early developmental stage. In contrast, the protein value (g AA digested in the small intestine per kg of DMI) was higher for pulp silage of grass fractionated twice compared with silage of chopped grass. Moreover, CH_4 yield was not affected by processing.

Silages

All silages were well preserved and appeared of good quality upon usage. The chemical composition of silages reflected the prolonged regrowth because grass harvested at late compared with early developmental stage had lower CP concentration and in vitro OMD

Table 8. Gas exchange in cows fed TMR based on silage of chopped grass and pulp silage of fractionated grass harvested at 2 developmental stages (n = 4)

	Treatment[1]							*P*-value[3]		
	Early			Late						
Item	GS	1×P	2×P	GS	1×P	2×P	SEM[2]	Dev	Pro	Dev × pro
CH_4										
L/d	591	588	555	639	611	577	28.3	0.06	0.07	0.76
L/kg of DMI[4]	32.3	34.3	35.1	37.3	37.0	36.8	1.24	<0.01	0.22	0.08
L/kg of ECM[4]	23.8	24.2	22.3	26.3	25.4	25.9	1.58	<0.01	0.47	0.29
CO_2, L/d	6,676^{x}	6,383^{x}	5,979^{y}	6,574^{x}	6,270^{x}	5,765^{y}	221.0	0.25	<0.01	0.91
CH_4:CO_2	0.088^{y}	0.092^{xy}	0.093^{x}	0.097^{y}	0.097^{xy}	0.100^{x}	0.0022	<0.01	0.05	0.48
O_2, L/d	6,023^{x}	5,706^{x}	5,352^{y}	5,828^{x}	5,583^{x}	5,095^{y}	188.7	0.05	<0.01	0.83
H_2, L/d	3.79^{x}	2.52^{y}	2.71^{y}	4.18^{x}	2.46^{y}	2.25^{y}	0.371	0.86	<0.01	0.40
RQ[5]	1.11	1.12	1.12	1.13	1.12	1.13	0.014	0.07	0.64	0.72

^{x,y}Values within the same row with different superscripts differ due to processing ($P \leq 0.05$).

[1]Treatments: Early = grass harvested at early developmental stage; Late = grass harvested at early developmental stage; GS = silage of chopped grass; 1×P = silage of pulp fractionated once; 2×P = silage of pulp fractionated twice.

[2]Highest standard error of the mean.

[3]*P*-values: Dev = developmental stage; Pro = processing; Dev × pro = interaction between developmental stage and processing.

[4]DMI and ECM yields were determined in the period where gas exchange was measured, and therefore differ from results reported in Tables 2 and 7.

[5]Respiration quotient = CO_2 produced divided by O_2 consumed.

(Table 1). In addition, iNDF proportion was higher, but only when comparing LGS to EGS.

Silages of chopped grass obtained DM concentrations lower than the 320 g/kg aimed for, which was mainly caused by the short wilting periods. Grass harvested for fractionation had an initial DM concentration of ~170 g/kg, and despite water was added to the pulp before the second fractionation, the DM concentration continued to increase compared with the first fractionation. The increase in DM concentration for each fractionation step was probably driven by disintegration of plant fibers during each fractionation, aiding the extraction of water. The reason why CP concentration was not affected by processing was probably that in addition to protein, other soluble compounds (carbohydrates) are extracted during fractionation (Kamm et al., 2016), also indicated by the more than 50% lower ash concentration in pulp silage of grass fractioned twice compared with silage of chopped grass. At comparable CP concentration as to our study, Damborg et al. (2018) showed no difference in CP concentration between the whole-plant material and the pulp after fractionation, and did also show that a considerably large part of CP remaining in pulp was associated with the fibers (NDF). This was in alignment with our study, where concentration and proportion of NDIN were higher for pulp silage of grass fractionated twice compared with silage of chopped grass (Table 1), and suggested that extraction of all CP by fractionation from the grass seems impossible. Savonen et al. (2020) and Sousa et al. (2022) reported considerably lower CP concentrations in pulp separated from grass silage compared with the whole-plant silage. This indicates that fractionation of fresh (as in our study) versus ensiled forages seems to affect the expected difference in CP concentration between the remaining pulp and the whole-plant material. However, the differences in CP concentration between pulp and whole plant probably also depends on the ensiling process itself because the ensiling process seems to increase the proportion of extractable protein. Therefore, direct comparison of effects from fractionating fresh or ensiled forage may be problematic. Within each developmental stage, the aNDFom concentration was higher for pulp silage compared with silage of chopped grass, and the concentration increased for each fractionation. This was due to fiber retention in the screw press during fractionation, and by each fractionation, an additional amount of soluble nutrients was extracted into the liquid fraction. The trend in aNDFom concentration related to processing within each developmental stage was probably the main factor contributing to the changes in OMD determined in vitro, when comparing silage of chopped grass to pulp silage.

The loss of easily fermentable carbohydrates during fractionation could have resulted in limited production of lactic acid in pulp silages, but pH was sufficiently low for all silages, and also lower for pulp silage compared with the silage of chopped grass (Table 1). Reduced concentrations of L-lactate and acetate in silages indicated that less acid was required to lower pH and stop fermentation, when comparing silage of chopped grass to pulp silage. However, the achieved levels of pH and acid could be related to the higher DM concentration (McDonald et al., 1991) and the lower buffer capacity (McDonald Henderson, 1962) of pulp silage compared with silage of chopped grass. The change in buffer capacity was likely caused by the changes in concentrations of ash (i.e., minerals) and soluble N as these are closely related to buffer capacity.

aNDFom Digestibility and Ruminal Kinetics

The apparent ruminal digestibility of aNDFom and DNDF (feed to ileum for both) was higher for L2×P compared with LGS (Table 3). Ruminal digestibility of NDF depends on the competitive processes of feed particles being either degraded or passing out of the rumen. The k_d of DNDF was expected to be higher for pulp compared with the chopped grass due to the intensive processing during fractionation. However, using ruminal evacuation data, we found no effect of processing on the k_d of DNDF (Table 4). For comparison, Damborg et al. (2019) found that the in situ determined k_d of potentially degradable NDF was 0.84 percentage-units higher in pulp silage compared with the whole-plant silage. In contrast to what we expected, k_d of DNDF was probably not different between treatments because of the parallel increase (38%) in the ruminal pool size of DNDF, when comparing GS to 1×P and 2×P, respectively. The parallel increased pool size of DNDF would dilute any possible increase in degradation of DNDF. Furthermore, our estimation of k_d of DNDF was based on ruminal evacuation data obtained at a single time point. Diurnal variations of the ruminal pool size of DM and nutrients are known to occur (Huhtanen et al., 2007), which, as addressed by Lund (2002), could potentially interact with our dietary treatments. However, a sensitivity analysis of the current data (not shown) comprising adjustment of the ruminal pool size demonstrated that a possible treatment-related diurnal variation in ruminal pool size resulting in an overestimated pool size of cows fed 2×P compared with GS was far from the only explanation for the lack of difference in k_d of DNDF between treatments. To obtain exactly equal k_d of DNDF for GS and 2×P within each developmental stage, ruminal pool size

of DNDF for E2×P and L2×P should have been 22 and 9% lower than observed, respectively, which we judge as being considerably more than expected treatment-related bias in average DNDF pool size.

Fractionation of the grass may have caused greater entanglement of pulp silage fibers and particles in the ruminal content. Despite lacking data to support these considerations, it is noteworthy that ruminal content from cows fed 2×P clearly seemed more densely packed with a paste-like appearance and less stratification of feed particles. Such conditions could possibly have affected fiber passage and degradation kinetics, but k_p of iNDF determined from ruminal evacuations was not affected by processing (Table 4). In addition, no significant effect of the interaction between developmental stage and processing on the DNDF:iNDF ratio of ruminal content was found, which would otherwise have been a possible explanation for the effect on apparent ruminal digestibility of aNDFom (feed to ileum) of processing at late but not early developmental stage (Lund et al., 2007).

Apparent total-tract digestibility of aNDFom was 68 g/kg higher for L2×P compared with LGS, whereas processing had no effect on apparent aNDFom digestibility at early developmental stage (Table 3). Higher apparent digestibility of hemicellulose seemed to be the main driver for this higher apparent total-tract aNDFom digestibility. Likewise, Damborg et al. (2019) observed a 95 g/kg greater apparent total-tract digestibility of aNDFom, when comparing pulp silage to the corresponding chopped whole-plant grass-clover silage. However, Damborg et al. (2019) used a forage, where clover constituted 55% on DM basis of the experimental silage compared with the only-grass forage in our experiment. The maceration of plant fibers during fractionation might affect legume fibers differently from that of grass fibers because lignin deposition occurs in the xylem of the vascular tissue and only in stems in legumes, whereas lignin is present in almost all tissues and organs in ryegrass (Buxton and Redfearn, 1997). The limited difference in stem proportion in the current study (75 and 115 g/kg on DM basis for early and late developmental stage, respectively) indicated that because processing affected apparent ruminal aNDFom digestibility (feed to ileum) only at late and not early developmental stage, fractionation may affect digestion of leaves as well as stems of forages. For comparison, apparent total-tract digestibility of NDF in the studies by Savonen et al. (2020) and Sousa et al. (2022) has shown not to be different between pulp of silage and the whole-plant silage, although silage and not fresh forage was used for fractionation. For application on farms, use of pulp as feed for ruminants includes estimation of fiber digestibility for feed ration planning. These results suggested that our understanding of the mechanisms of fiber digestion is not unequivocal when comparing pulp to the whole plant. Thus, further research is needed for quantifying the effect of fractionation on the physical properties of the feed particles of pulp and the subsequent variation in effect on fiber digestibility.

When comparing pulp silage to the whole-plant silage, Damborg et al. (2019) observed contradicting effects on OMD depending on the technique used for determining digestibility; hence, in vitro showed lower OMD of pulp silage, whereas in vivo showed higher apparent total-tract digestibility of OM of pulp silage compared with the whole-plant silage. In our study, compared with silage of chopped grass, the in vitro determined OMD was lower for pulp silage of grass fractionated once, and it was even lower in pulp silage of grass fractionated twice (Table 1). In the current study, an interaction indicated that E2×P had lower apparent total-tract digestibility of OM than E1×P, whereas no differences between other treatments were found (Table 3). Some mismatch between estimates obtained from in vitro and in vivo techniques therefore still seem to occur, when comparing effects of physical processing (i.e., fractionation using a screw press in our experiment) on digestibility. One potential explanation could be the already mentioned masking effect of laboratory processing (drying and grinding) of fractionation effects on digestibility (Damborg et al., 2019).

The concentration of aNDFom in feed has a greater filling effect than other nutrients, and is therefore negatively correlated with voluntary DMI (Allen, 1996). However, the ruminal pool size of DM, aNDFom, and iNDF were higher in 2×P compared with GS, which indicated that voluntary DMI was not only physically regulated, or that physical regulation changed when feeding pulp silage compared with silage of chopped grass, or that physical fill depends on more than aNDFom (Table 5). The same increase in ruminal pool of aNDFom and decrease in DMI was observed, when grass silages of increasing maturity stages were fed (Rinne et al., 2002). Based only on the fact that aNDFom concentration of diets was 140 g/kg of DM higher in 2×P compared with GS (data not shown), DMI was expected to be more different than what was observed due to higher fill. However, as indicated by Damborg et al. (2019), where similar DMI but higher NDF digestibility were reported when comparing pulp silage to the whole-plant silage, factors including particle size and particle fragility might also have affected the fill of pulp differently than chopped grass (Allen, 1996). Microscope photos of the 6 silages indicated that fractionation affected those factors, which subsequently might have had limited the reduction in the fill value and resulted in the limited difference in DMI when

comparing GS to 2×P. However, a direct measurement of the silage particle sizes were not acquired in the current experiment, but would probably have aided the understanding of the connection between processing and digestion.

Protein Digestibility and Metabolizable Protein

The amount of AA digested in the small intestine per kg of DMI was higher for 2×P compared with GS, whereas processing had no effect on the amount of AA digested in the small intestine in g/d (Table 4). The AA digested in the small intestine (i.e., MP supply) originates from feed AA not digested in the rumen, microbial AA, and endogenous AA. The effect of processing on true ruminal digestibility of CP at early and not late developmental stage could probably be attributed to the larger difference in concentration of NDIN between the pulp silage of grass fractionated twice and silage of grass at early compared with late developmental stage. These in vivo data (EGS vs. E2×P) were in alignment with previous in situ data (Damborg et al., 2018), where effective CP degradation in the rumen was also lower in pulp compared with the whole plant, but not to the same extent as in our in vivo study. In the current study, 2×P had lower concentrations of ammonia N in ruminal fluid than 1×P. The reason for this was unknown, but all estimates were within biological range of ammonia N concentration in the rumen (Abdoun et al., 2006).

The effect of processing on the duodenal flow of AA of feed and endogenous origin depended on developmental stage, and the changes within developmental stage were likely related to the changes in true ruminal CP digestibility (Table 3). The highest duodenal flow of AA of feed and endogenous origin was found for E2×P, which also had the lowest true ruminal CP digestibility. Interestingly, NDIN concentration (g/kg of aNDFom; data not shown) was higher for pulp silage of grass fractionated twice compared with silage of chopped grass, both harvested at early developmental stage. This suggested that some Maillard reactions may have occurred, possibly during fractionation, and that this likely contributed to the lower true ruminal CP digestibility and higher duodenal flow of AA of feed and endogenous origin in E2×P compared with EGS. The duodenal flow of microbial AA was numerically slightly higher for GS, and differed only significantly from 1×P and not 2×P, and this was probably caused by the higher aNDFom concentration (i.e., slowly digested carbohydrates) in 1×P and 2×P compared with GS. However, that does not explain why no difference between 1×P and 2×P was found in duodenal flow of microbial AA. All combined, the changes in duodenal flow of AA of feed, endogenous, and microbial origin combined with the apparent small intestinal digestibility of AA, which did not differ between treatments, resulted in improved protein value (g AA digested in the small intestine per kg DMI). This was in agreement with Damborg et al. (2018), who also showed higher in situ determined protein value of pulp from fractionated ryegrass, white clover, red clover, and lucerne compared with the whole plant of each forage. The total amount of AA digested in the small intestine (g/d) was not affected, although DMI was lower for both 1×P and 2×P compared with GS. Moreover, the CP concentration of TMR was lower for TMR with forage harvested at late compared with early developmental stage, while DMI and CP intake was lower for 2×P compared with GS. Therefore, when flows were expressed in g/kg of DMI, data were confounded by the lower CP intake at similar DMI for TMR with forage harvested at late compared with early developmental stage.

We used cows in mid-lactation and all cows had a sufficient supply of MP. Therefore, no further improvements of the milk production was expected from feeding pulp silage compared with silage of chopped grass, despite it had a greater protein value (i.e., higher MP; Huhtanen and Hristov, 2009). Feed conversion ratio (kg ECM/kg of DMI) was higher for 2×P compared with GS, but experimental periods lasted only for 21 d and results should therefore be interpreted with care (Table 7).

Production of CH_4

We expected CH_4 yield (L/kg of DMI) to be higher for 2×P compared with GS and higher for rations with silages harvested at late compared with early developmental stage due to the greater amount of aNDFom digested in the rumen. Fermentation of aNDFom in the rumen yields primarily acetate with H_2 surplus, and H_2 is used by methanogens forming CH_4 (Boadi et al., 2004). Indeed, CH_4 yield was higher for cows fed silage harvested at late compared with early developmental stage, whereas no effect of processing on CH_4 yield or intensity was detected (Table 8). Interestingly however, although the amount of aNDFom degraded in the rumen (feed to ileum) and the proportion of acetate in ruminal fluid were higher (Table 4 and 6), the daily production of H_2 was lower in 2×P compared with GS. In Hellwing et al. (2018), heifers were fed silages originating from the same experiment as Damborg et al. (2019), and they concluded that CH_4 yield was higher when feeding pulp silage compared with the whole-plant silage (30.9 vs. 29.2 L/kg DMI,

respectively). In contrast to our study, Hellwing et al. (2018) found that the proportion of acetate was lower and the proportion of butyrate was higher, when feeding pulp silage compared with the corresponding grass-clover silage. To our knowledge, no other in vivo experiments measuring production of CH_4 using lactating cows fed with pulp have been conducted.

CONCLUSIONS

Feeding rations with pulp silage of grass fractionated twice compared with silage of chopped grass resulted in higher apparent total-tract digestibility of aNDFom, when grasses were harvested at late developmental stage. Moreover, feeding rations with pulp silage of grass fractionated twice compared with silage of chopped grass resulted in higher protein value (g AA digested in the small intestine/kg of DMI), but had no effect on CH_4 yield (L/kg of DMI) or k_d of DNDF. Feeding pulp silage of grass fractionated once generally yielded results intermediate to cows fed silage of chopped grass and pulp silage of grass fractionated twice. The results indicated that pulp silage of grass from green biorefining can be a valuable feedstuff for dairy cows, but the change in feeding value by the wet fractionation depends on the developmental stage of grass during harvest.

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