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Effects of partial replacement of grass silage with maize silage on feed intake, enteric methane emissions and milk production in dairy cows in northern conditions

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We conducted three experiments to investigate the effects of partial replacement of grass silage with maize silage (25% or 50% of the dry matter [DM]) on feed intake, milk production, diet digestibility, and methane (CH_4) emissions in dairy cows. The starch concentrations of maize silages were high for northern latitudes and ranged from 236 g kg^{-1} DM at early harvest to 278 and 254 g kg^{-1} DM at late harvest, respectively. Partial replacement of grass silage with maize silage increased feed intake, whereas diet digestibility decreased. Milk and energy corrected milk (ECM) yields increased in one experiment, whereas no corresponding changes were observed in the other experiments. Replacing grass silage with maize silage improved nitrogen utilization in all the experiments and CH_4 intensity (g kg^{-1} ECM) decreased in one experiment. In northern conditions, the beneficial effects of maize silage on feed intake can be achieved, while effects on milk production and enteric CH_4 emissions are less consistent due to annual variation in maturity and starch content of maize.

Key words: milk yield, methane, forage, digestibility, *Zea mays* L.

Introduction

In recent decades, grass silage has been used as a major forage source in feeding of dairy cows in northern Europe. The cultivation of forage maize, which is commonly used in temperate and tropical areas, has not been possible due to the cool and unfavorable climate. In northern regions of temperate zone, short growing season, low effective temperature sum (ETS), and early frost are limiting factors for maize growth and maturation (Pulli et al. 1979). However, the cultivation of maize for silage has increased in recent years due to the development of new, early varieties and cultivation methods, as well as prolonged growing season because of climate change (Eckersten et al. 2012). Maize has an advantage over grass by producing a large amount of dry matter (DM) in a single harvest. Replacing grass silage with maize silage may also reduce enteric CH_4 emissions in dairy cows (Brask et al. 2013, van Gastelen et al. 2015).

Due to the short growing season, forage maize often does not have enough time to develop sufficiently in northern conditions (Mussadiq et al. 2012). Starch is the major source of metabolizable energy (ME) in maize silages (Khan et al. 2015), and a long enough growing season is a prerequisite for starch to accumulate in the maize ears. Maize silages ensiled at a very early stage have typically low starch content and starch/neutral detergent fibre (NDF) ratio resulting in lower dry matter intake (DMI) and milk yield (Khan et al. 2015). Seleiman et al. (2017) found that the starch content of forage maize doubled as the growing season was extended from 120 to 150 days in Southern Finland (60° N). As the growing season lengthens, the DM and starch content of forage maize generally increases, reflecting the progressing maturity of the maize (Khan et al. 2015). However, DM content depends on the growing site, growth conditions, and variety, and is therefore not a reliable indicator of crop maturity on its own (Mussadiq et al. 2012, Seleiman et al. 2017).

Maize silage is often used to partially replace grass silage. The effect of maize silage on feed intake and milk production in dairy cows depends on the proportion of maize silage in the diet and the composition and feed value of both maize and grass silages. In a study by Keady et al. (2008), replacing 40% grass silage with maize silage improved feed intake more when the digestibility of grass silage was low. However, replacing grass silage by maize silage with moderate starch content may decrease digestibility of the diet when compared with high-digestibility grass silage (O'Mara et al. 1998). According to a meta-analysis by Khan et al. (2015), the positive effects of maize silage on feed intake, milk production, and milk protein content are greatest when the DM content of maize

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silage is 300–350 g kg^{-1}, corresponding to starch content of 275–319 g kg^{-1}. Milk yield and protein concentration responses were strongly positively correlated to the starch/NDF ratio of maize silage.

Partially replacing grass silage with maize silage can reduce CH_4 formation in the rumen of dairy cows (Dewhurst 2013). Adding maize silage increases the starch content and reduces the fiber content of the diet, which can increase the proportion of propionate among the volatile fatty acids formed in the rumen. Van Gastelen et al. (2015) observed that complete replacement of grass silage by maize silage reduced CH_4 yield (g CH_4 kg^{-1} DMI) by 11% and CH_4 intensity (g CH_4 kg^{-1} fat- and protein-corrected milk) by 8%. However, the capacity of maize silage to reduce CH_4 emissions depends on the maturity of maize, and hence it is related to DM and starch contents of maize (Hatew et al. 2016).

We aimed to investigate the effect of partial replacement of grass silage with maize silage produced at northern latitude beyond 60° on animal performance, diet digestibility, nitrogen utilization, rumen fermentation, and ruminal CH_4 emissions in dairy cows. We chose to study partial replacement, which is generally recommended according to the review by Khan et al. (2015). They concluded that a mixture of grass and maize silage improves animal performance compared to grass or maize silage as a sole forage. In addition, we aimed to study the effect of maize maturity on the crop's nutritive value at two different maturity stages. We hypothesized that partial replacement of grass silage with maize silage would increase DMI and milk yield and decrease DM digestibility and ruminal methanogenesis. Further, we hypothesized that earlier harvest would decrease starch content of the maize silage and reduce positive effects on milk yield and CH_4 emissions.

Materials and methods

Animals, housing, and treatments

Three experiments were conducted at the University of Helsinki Viikki research farm (60° 13´ N, 24° 02´ E) in Helsinki, Finland. In all experiments, the effect of partial replacement of grass silage with maize silage on feed intake, milk production, and diet digestibility was studied. One of the experiments focused on rumen fermentation, and two experiments on CH_4 emissions. The experiments were approved by the Project Authorisation Board (Hämeenlinna, Finland). All experimental procedures were conducted following the guidelines established by the European Union Directive 2010/63/EU and the current Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific or Educational Purposes 497/2013). Animal health and welfare were monitored throughout the experiments.

In EXP1, we used nine multiparous (from second to fourth lactation) Ayrshire cows housed in tie stalls, averaging (± SD) 69 ± 17 days in milk (DIM), 674 ± 17 kg of body weight (BW), 3.35 ± 0.47 of body condition score (BCS), and 42 ± 5.8 kg milk yield, at the beginning of the trial. A replicated 3 × 3 Latin square design was used as the experimental model. Six of the cows had rumen cannulas (squares 1 and 2). Within squares animals were randomly allocated to the sequences of treatments. Each experimental period consisted of two weeks of adaptation, followed by measurements and sample collection in the third week. The experimental treatments were 100% first-cut grass silage (Grass), a mixture of 75% (DM basis) grass silage and 25% maize silage (Maize25), and a mixture of 50% grass silage and 50% maize silage (Maize50).

We used reversal (switch-back) design in EXP2 and EXP3, consisting of three four-week periods, and the same sequence of treatments for all the cows. The experimental treatments during periods 1 to 3 were 1) a mixture of 50% (DM basis) grass silage and 50% maize silage (Maize50); 2) 100% grass silage (Grass) and 3) Maize50. The whole herd of Ayrshire cows in a free-stall barn with an automated milking system was fed the experimental diets. There were on average 41.2 and 40.3 cows in the free-stall during EXP2 and EXP3, respectively. Average milk yields of the herd were 10195 and 11227 kg ECM per cow in years 2020 and 2021, when the experiments were conducted. In each experiment, 10 multiparous cows were used for milk and faecal sample collection. We aimed at forming homogenous group of cows by selecting post-peak lactation cows based on milk yield, parity, and DIM. In EXP2, the cows were from second to fifth lactation, averaging (± SD) 107 ± 25 DIM, 731 ± 72 kg of BW, 3.46 ± 0.40 of BCS, and 44 ± 5.5 kg milk yield. In EXP3, the cows were from second to sixth lactation, averaging (± SD) 76 ± 39 DIM, 743 ± 99 kg of BW, 3.29 ± 0.30 of BCS, and 46 ± 9.3 kg milk yield at the beginning of the trial.

Experimental feeds and diets

Experiments 1 and 2 were conducted simultaneously from January to April in 2020, using the same maize silage harvested as late as possible to maximize starch and DM contents, whereas EXP3 was conducted one year later from January to April in 2021, using maize silage ensiled at an earlier stage of maturity. The forages used in the experiments were harvested from the fields of Viikki research farm. Grass silages consisted of mixtures of timothy grass (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). The maize (*Zea mays L.*; Pioneer P7326; FAO 180; Pioneer Hi-Bred International, Johnston, USA) was sown under mulch film (Samco Degradable Film, Samco, Ireland). The weather data for both years with monthly mean temperatures and precipitation is presented Supplemental Table 1.

The grass silage for EXP1 was from first cut in June 2019 and ensiled after pre-wilting using Krone XXL R/GL forage wagon (Krone GmbH, Spelle, Germany). In EXP2 grass silage from the same cut was ensiled after pre-wilting in round bales using Lely Welger RPC 245 Tornado round baler (Lely International, Maassluis, Netherlands). The silage additive used (5 l ton^{-1} of feed) was AIV2 Plus Na (Taminco Finland Oy, Eastman Chemical Company, Oulu, Finland). The maize used in EXP1 and EXP2 was sown on May 9, 2019, and harvested after a 151-d-growing period on October 7, 2019 using Claas Jaguar 950 forage harvester (Claas KGaA GmbH, Harsewinkel, Germany). The ETS (°Cd) for maize between sowing and harvest dates was calculated with a +10 °C base temperature (Liimatainen et al. 2022), and it was 868 °Cd. The maize silage was preserved in a bunker silo using Kofasil Ultra K as an additive (Addcon GmbH, Bitterfelg-Wolfen, Germany) at a rate of 4 l ton^{-1} of feed.

The grass silage for EXP3 was harvested from first cut in June 2020 and ensiled in a bunker silo. Harvesting and ensiling methods were the same as in EXP1 and EXP2. The silage additive was applied at a rate of 4.7 l ton^{-1} of feed. The maize was sown on May 22, 2020, and it was harvested on September 24, 2020, after a 125-d growing period (ETS 805 °Cd). It was harvested and ensiled with the methods described for EXP1 and EXP2, using an additive (AIV 2000 Plus Na, Taminco Finland Oy, Eastman Chemical Company, Oulu, Finland) at a rate of 4 l ton^{-1} of feed.

In all experiments, partial mixed ration (PMR) was fed *ad libitum* and distributed three times a day. The forage-to-concentrate ratio in the PMR was 65:35 on a DM basis. In EXP1 and EXP2, the concentrate mixture in the PMR contained barley (51% of the concentrate mix DM), oats (15%), faba beans (20%), peas (5%), molassed sugar beet pulp (5%), minerals and vitamins (Seleeni-E-Melli TMR, Lantmännen Feed Ltd, Turku, Finland; 3%), and propylene glycol (1%). In EXP3, the concentrate mixture in the PMR consisted of barley (52% of the concentrate mix DM), oats (17%), faba beans (22%), molassed sugar beet pulp (5%), minerals and vitamins (3%), and propylene glycol (1%). In addition to the PMR, the cows were given commercial supplementary feed containing rapeseed meal (42%), oats (15%), barley (15%), and molasses (8%) as major ingredients (Hankkija Oy, Hyvinkää, Finland). The supplementary concentrate was distributed three times a day (600 h, 1200 h, and 1700 h) to feeding bins in EXP1, while in EXP2 and EXP3, the cows received the supplementary feed during milking in the automated milking system. Mean intake was 6.1 kg DM/d, 6.6 kg DM/d, and 7.0 kg DM/d in EXP1, EXP2 and EXP3, respectively. Average dietary concentrate proportions (DM basis) were 51.0% (Grass), 50.2% (Maize25), and 49.8% (Maize50) in EXP1; 50.6% (Grass) and 51.6% (Maize50) in EXP2; and 52.9% (Grass) and 52.8% (Maize50) in EXP3. The cows had free access to drinking water and salt blocks.

Sample collections and analysis

In EXP1, the cows were milked twice a day using a pipeline milking machine (DeLaval, Tumba, Sweden) starting at 600 h and 1700 h. Milk production was measured for each milking using a Tru-Test milk meter (WB Auto Sampler, Tru-Test, Auckland, New Zealand). Milk samples were collected from each cow during the last week of each period, from four consecutive milkings. In EXP2 and EXP3, the cows were milked using an automatic milking system (Lely Astronaut A3, Lely International, Maassluis, Netherlands). Milk samples were collected using a Lely Shuttle sampling device from each cow during the last week of each period. The samples were collected for three consecutive days at each milking in EXP2, and for two consecutive days in EXP3. The number of samples per cow per period ranged from 4 to 10 in EXP2, and from 4 to 7 in EXP3. In all experiments, samples were preserved with Bronopol preservative (Valio Ltd., Helsinki, Finland) and sent to a commercial laboratory (Valio Ltd., Seinäjoki, Finland) for mid-infrared analyses (MilkoScan FT+; Foss Electric A/S, Hillerød, Denmark) for fat, crude protein (CP), lactose, and urea.

The consumption of PMR was measured using an automatic feed intake monitoring system (RIC, Roughage Intake Control, Insentec, Marknesse, Netherlands) in all experiments. Representative samples of the experimental forages and concentrate mixtures were collected daily during the final week of each period and stored at –20 °C until they were composited by period and dried at 50 °C for 48 h for subsequent feed analysis. Separate samples of fresh forages were stored at –20 °C for analysis of fermentation quality. For the commercial concentrates, samples were taken once per period. Faecal spot samples (0.5 l) were collected from the rectum twice daily at 0800 h and 1500 h during the final week of each period to determine the apparent total-tract digestibility of the diet, using acid indigestible acid (AIA) as an internal marker (Van Keulen and Young 1977). Samples were composited per animal and period and stored at −20 °C. After each period, composited samples were defrosted, mixed thoroughly, dried in a forced air oven (100 °C for 1 h and thereafter 70 °C for 48 h) and ground through a 1.5-mm screen for the analysis chemical composition. Separate fresh faecal subsamples were taken from composited samples and stored at −20 °C for the determination of CP content.

Dry matter content of the feed and faecal samples was determined by oven drying (103 °C, 24 h) and DM of the forages was corrected for volatile losses according to Huida et al. (1986). The ash content of the samples was determined by ashing at 600 °C for 24 h (Heraeus Thermicon T; Heraeus, Hanau, Germany). Neutral detergent fiber was analyzed according to Van Soest et al. (1991) with FiberTherm FT12 analyzer (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and reported in ash-free basis. Heat-stable α-amylase was used in the NDF analysis of concentrate components and maize silages. Crude protein content was determined with the Kjeldahl method (AOAC International 1995) and AIA by acid hydrolysis (Van Keulen and Young 1977). Total fat was analyzed by petroleum ether extraction and HCl hydrolysis (SoxCap 2047 Hydrolysis Unit, Foss Soxtec 8000; Foss Analytical, Hillerød, Denmark). Starch content was measured by amyloglucosidase and α-amylase method with K-TSTA kit (Megazyme, Co. Wicklow, Ireland) and spectrophotometer (Shimadzu UV-VIS mini 1240, Shimadzu Europa GmbH, Duisburg, Germany), using version c of manufacturer's operating instructionwith preceding wash with ethanol as instructed in the version e paragraphs 1–5 (AOAC method 996.11). Silage lactic acid and VFA concentrations were determined by ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC, Waters, Milford, USA), as described in detail by Puhakka et al. (2016). Water-soluble carbohydrates (Somogyi 1945, Salo 1965), and NH_3-N (McCullough 1967) concentrations were analyzed using colorimetric methods and a spectrophotometer (Shimadzu UV-VIS mini 1240), and ethanol concentration by an enzymatic kit (cat. no. 176290, R-Biopharm AG, Darmstadt, Germany). The silage OM *in vitro* digestibility (D-value) was determined as described by Liimatainen et al. (2022). The indigestible neutral detergent fiber concentration was determined by incubating nylon bags (pore size of 17 µm) in the rumen of two dairy cows fed grass silage–based diets for 12 d according to Ahvenjärvi et al. (2000). The concentration of non-fibre carbohydrates (NFC) was calculated by subtracting the concentrations (g kg^{-1} DM) of NDF, CP, total fat, and ash from 1000.

Samples of rumen fluid were collected in EXP1 on d 20 of each period from six rumen-cannulated cows at 1.5 h intervals starting before morning feeding (at 0530 h, 0700 h, 0830 h, 1000 h, 1130 h, 1300 h, 1430 h and 1600 h). Samples (100–150 ml) were collected via rumen cannula from the mid-ventral region of the rumen using a vacuum pump. Rumen fluid was filtered through a single layer of cheesecloth and pH was immediately measured with electronic pH meter (S20 SevenEasy pH, Mettler-Toledo Ltd, Leicester, Great Britain). For the determination of VFA concentrations, a subsample of 5 ml of ruminal fluid was preserved with 0.5 ml of saturated mercury (II) chloride and 2 ml of 1 mol l^{-1} sodium hydroxide and stored at −20 °C. Subsamples of ruminal fluid (15 ml) for the determination of NH_3-N were preserved with 0.3 ml of 9 mol l^{-1} sulphuric acid and stored at −20 °C. Volatile fatty acids and NH_3-N concentrations were determined as described above for feed samples. A detailed description of the preparation of rumen fluid samples for VFA determination was presented by Lamminen et al. (2017).

In EXP2 and EXP3, CH_4 emissions from cows were measured during the last week of each period using the GreenFeed system (C-Lock Inc, Rapid City, USA) installed in the milking robot as described by Huhtanen et al. (2015). Automatic calibrations using a mixture of nitrogen (N_2) and oxygen (O_2), and a mixture of CH_4, O_2, hydrogen (H_2), and carbon dioxide (CO_2) were conducted daily. The CO_2 recovery tests were performed at the beginning of the experiment and before the last week of each period following the manufacturer's recommendations. Average (± SD) CO_2 recovery was 103.9 ± 1.18% in EXP2 and 98.3 ± 2.82% in EXP3. Air flow of the system was monitored daily, with the aim to keep the air flow above 25 l s^{-1}. A minimum of 10 successful visits (at least 2 min of uninterrupted measurement) per cow per sampling period was used as a criterion for including the cow in the CH_4 data. In EXP2, to increase the low number of observations fulfilling the measurement criteria, CH_4 data were supplemented with three cows receiving the same diets as the experimental cows. These cows had the same feeding as the cows originally selected for sample collection, and their feed intake, milk yield and milk composition were recorded. The number of cows used for CH_4 emission data were 9, 8 and 7 for periods 1 to 3 in EXP2, and 10, 10 and 8 for periods 1 to 3 in EXP3.

Calculations and statistical analysis

Feed intake and milk yield of the cows were recorded daily throughout the experiments, but only the measurements on the last week of each period were used for statistical analysis. Energy-corrected milk yield (ECM) was calculated according to Sjaunja et al. (1991). The ME, metabolizable protein (MP), and protein balance in the rumen (PBV) were calculated according to the Finnish Feed Tables and Nutrient Requirements (Luke 2024).

In EXP2, one cow suffered from hoof problems in period 3 and her data were not used for any analyses from that period. In EXP3, one cow suffered from unspecified health problems and inappetence in period 3 and her data were not used for any analyses from that period.

The statistical analysis of the results was performed using analysis of variance (ANOVA) with the Mixed procedure of SAS software (version 9.4, SAS Institute Inc., Cary, USA). In the statistical analysis of feed intake, milk production, apparent total tract digestibility, and feed utilization (ECM, kg / DMI, kg) data of EXP1, the fixed factors in the model were treatment, square and period within square, while animal was treated as a random factor. Repeated measurement analysis was used for rumen fermentation data. The model included treatment, sampling time, interaction between treatment and sampling time, square and period within square as fixed factors, and animal was treated as a random factor. The linear and quadratic effects of the increasing proportion of maize silage in the diet were examined using polynomial contrasts.

In the analysis of EXP2 and EXP3, period (i.e. treatment effect) was a fixed factor and animal was treated as a random factor. The linear and quadratic effects of the period were examined using polynomial contrasts. The linear effect represents the effect of time, while the quadratic effect represents the effect of forage. Before analysis, the normality of the residuals was tested using the Univariate procedure of SAS with the Shapiro-Wilk test. If the absolute value of the residual was greater than 3, the observation was deemed as an outlier and removed from statistical analysis. Treatment effects were considered significant at $p < 0.05$ and tendencies at $0.05 \le p < 0.10$. All data are presented as least square means.

Results

Chemical composition of silages

We observed distinct differences in the chemical composition of grass and maize silages used in the three experiments. Ash, NDF and CP contents were consistently lower, and NFC and starch contents were greater in maize silage compared with grass silage. Also, the DM and starch contents of the late-harvested maize silages (EXP1 and EXP2) were greater than those of the early-harvested in EXP3 (Table 1). In EXP3, the DM content of grass silage was low compared to maize silage.

Table 1. Chemical composition of maize and grass silages

	Exp. 1		Exp. 2		Exp. 3	
Item	Maize	Grass	Maize	Grass	Maize	Grass
Dry matter, g kg^{-1}	359	316	351	361	287	226
Chemical composition (g kg^{-1} DM)						
Ash	39.5	83.1	40	79.3	48.4	99.6
Crude protein	78.9	131	79.1	126	64.9	156
Total fat	18	21.5	15.9	19.7	18.6	26.1
NDF[1]	400	586	390	590	420	541
iNDF[2]	96.1	103	99.3	116	102	75.1
NFC[3]	464	179	475	185	448	177
Starch	278	1.7	254	4.1	236	1.5
D-value[4]	679	652	676	657	672	635
MP[5]	77.2	77.3	77.2	77.4	74.5	78.5
PBV[5]	-37.7	14.8	-37.5	10.4	-48.3	39.6
ME[5], MJ kg^{-1} DM	10.5	10.4	10.5	10.5	10.4	10.2

[1] NDF = Neutral detergent fibre; [2] iNDF = Indigestible neutral detergent fibre; [3] NFC = Non-fibre carbohydrates; [4] D-value = digestible organic matter in DM; [5] Metabolizable energy (ME), metabolizable protein (MP), and protein balance in the rumen (PBV) were calculated according to the Finnish feed evaluation system (Luke 2024) for rumen degradable protein fractions and energy content.

In terms of pH, fermentation acids and water-soluble carbohydrates (WSC), maize and grass silages showed relatively similar characteristics within EXP1 and EXP2 (Supplemental Table 2). In EXP3, grass silage had a greater content of fermentation products and a lower content of WSC than maize silage. The chemical composition of concentrate mixtures and compound feeds used in different experiments were close to each other (Supplemental Table 3).

Feed and nutrient intake and apparent total-tract digestibility

Partial substitution of grass silage with maize silage linearly increased DM, MP, starch and ME intakes of the cows ($p < 0.001$) in EXP1 (Table 2). Simultaneously, NDF ($p < 0.001$) and crude protein ($p = 0.008$) intakes, as well as PBV decreased linearly ($p < 0.001$). In EXP2 and EXP3, DM and starch intakes decreased, and NDF (only in EXP2) and CP intakes increased when the cows were shifted from feeding containing 50% maize silage to grass silage feeding (quadratic effect, $p < 0.001$) (Tables 3 and 4). In EXP3, the ME and MP intakes were lower (quadratic effect, $p = 0.002$) on grass silage- than on maize-silage-based diets (Table 4), and a similar tendency was observed in EXP2 (quadratic effect, $p = 0.08$ and $p = 0.06$ for ME and MP intake, respectively) (Table 3). The PBV was greater in EXP2 and EXP3 on grass silage-based diets (quadratic effect, $p < 0.01$).

As the proportion of maize silage increased, the digestibility of DM, NDF, and CP decreased linearly ($p < 0.01$) in EXP1. Correspondingly, DM, NDF, and CP digestibility were lower in EXP2 and EXP3 during the periods, when the cows consumed maize silage (quadratic effect, $p < 0.001$).

Table 2. Effects of partial replacement of grass silage with maize silage on feed intake, diet digestibility, and lactation performance in experiment 1

	Treatments[1]				Probability	
	Grass	Maize25	Maize50	SEM[2]	Linear	Quadratic
Intake, kg d^{-1}						
Dry matter	24.4	25.3	26.0	0.65	< 0.001	0.44
NDF[3]	9.37	9.17	8.86	0.258	< 0.001	0.44
Crude protein	4.01	3.97	3.89	0.094	0.008	0.65
Starch	3.87	4.96	5.98	0.130	< 0.001	0.58
MP[4]	2.30	2.38	2.44	0.072	< 0.001	0.45
PBV[5], g	589	413	244	15.9	< 0.001	0.86
ME[6], MJ d^{-1}	261	270	276	6.2	< 0.001	0.41
Digestibility, g kg^{-1}						
Dry matter	688	682	663	6.2	0.007	0.30
NDF	505	462	377	14.7	< 0.001	0.17
Crude protein	660	635	608	5.5	< 0.001	0.86
Starch	991	992	991	0.7	0.52	0.31
Milk production, kg d^{-1}						
Milk	32.8	33.3	34.0	1.44	0.09	0.83
ECM[7]	33.2	33.6	35.3	1.43	0.01	0.31
Milk composition, g kg^{-1}						
Fat	40.9	40.7	43.2	0.85	0.01	0.07
Protein	35.2	35.0	35.1	0.65	0.77	0.41
Lactose	45.0	45.2	45.1	0.53	0.50	0.21
Urea, mg dl^{-1}	22.3	19.5	17.4	0.83	< 0.001	0.55
Feed utilization						
Feed efficiency[8]	1.36	1.33	1.36	0.035	1.00	0.16
N utilization[9]	0.286	0.292	0.306	0.0083	0.02	0.48

[1] Forages in different proportions: Grass = 100% grass silage; Maize25 = 25% of grass silage DM replaced by maize silage; Maize50 = 50% of grass silage DM replaced by maize silage; [2] Largest SEM reported; n = 27 (n represents the number of observations used in the statistical analysis). Data are presented as least square means; [3] NDF = neutral detergent fibre; [4] MP = Metabolizable protein; [5] PBV = protein balance in the rumen; [6] ME = Metabolizable energy; [7] ECM = Energy corrected milk; [8] Energy corrected milk, kg/Dry matter intake, kg; [9] N milk/N intake

Milk production and composition

Replacing grass silage with maize silage tended to increase ($p = 0.09$) milk yield in EXP1, and linearly increased ECM ($p = 0.01$) (Table 2). The addition of maize silage tended to have a curvilinear effect ($p = 0.07$) on milk fat concentration, with the highest fat content observed when 50% of the grass silage was replaced by maize silage. Milk urea concentration decreased linearly with increasing proportions of maize silage in the diet ($p < 0.001$).

Milk yield decreased over time during EXP2 and EXP3, and we observed a similar trend for ECM yield in EXP2 (linear effect, $p < 0.05$) (Tables 3 and 4). However, no quadratic effects were found, indicating the absence of differences between treatments in milk and ECM yield. In EXP 2, milk protein, fat, and lactose yield decreased over time (linear effect, $p < 0.05$), but we did not find a similar effect in EXP3. Milk urea concentration was lower during the periods with maize silage feeding (quadratic effect, $p < 0.001$ in EXP2 and EXP3). In EXP3, milk fat concentration decreased (quadratic effect, $p = 0.02$) and fat yield tended to decrease (quadratic effect, $p = 0.07$) with the inclusion of maize silage in the diet. Forage source did not affect milk protein yield in either of the experiments (quadratic effect, $p > 0.10$).

Table 3. Effects of partial replacement of grass silage with maize silage on feed intake, diet digestibility, lactation performance, and gas emissions in experiment 2

	Treatments[1]				Probability	
Item	Maize50	Grass	Maize50	SEM[2]	Linear (Time)	Quadratic (Forage)
Intake, kg d^{-1}						
Dry matter	26.8	25.7	26.4	0.65	0.39	0.040
NDF[3]	8.80	9.70	8.87	0.230	0.67	< 0.001
Crude protein	4.13	4.23	3.88	0.101	0.002	0.001
Starch	6.01	4.11	6.03	0.135	0.86	< 0.001
MP[4]	2.55	2.43	2.47	0.061	0.07	0.06
PBV[5], g	277	584	210	13.6	< 0.001	< 0.001
ME[6], MJ d^{-1}	286	275	279	6.5	0.17	0.08
Digestibility, g kg^{-1}						
Dry matter	647	678	625	7.2	0.01	< 0.001
NDF[7]	353	498	319	10.0	0.002	< 0.001
Crude protein[7]	633	669	569	9.0	< 0.001	< 0.001
Starch[7]	987	985	988	1.0	0.38	0.04
Milk production, kg d^{-1}						
Milk	40.1	37.3	34.6	1.57	< 0.001	0.95
ECM	39.5	37.4	35.4	1.82	0.004	0.94
Milk composition, g kg^{-1}						
Fat	39.1	39.8	40.7	1.34	0.21	0.99
Protein	34.2	35.4	36.8	0.99	< 0.001	0.65
Lactose	45.1	44.8	44.8	0.47	0.20	0.30
Urea, mg dl^{-1}	19.9	28.4	23.5	0.90	0.003	< 0.001
Feed utilization						
Feed efficiency[8]	1.47	1.45	1.34	0.051	0.007	0.22
N utilization[9]	0.33	0.31	0.33	0.012	0.74	0.01
Gas emissions[10]						
CH_4, g d^{-1}	460	483	465	15.8	0.71	0.11
CH_4, g kg^{-1} DMI	17.3	18.6	17.2	0.63	0.88	0.02
CH_4, g kg^{-1} ECM	11.5	12.1	12.8	0.81	0.12	0.98
CO_2, g d^{-1}	13268	13670	13359	319.2	0.69	0.07
H_2, g d^{-1}	2.01	1.88	1.93	0.159	0.62	0.53

[1] Forages in different proportions: Grass = 100% grass silage; Maize50 = 50% of grass silage DM replaced by maize silage [2] Largest SEM reported; n = 29 unless otherwise stated. Data are presented as least square means; [3] NDF = neutral detergent fibre; [4] MP = Metabolizable protein [5] PBV = Protein balance in the rumen; [6] ME = Metabolizable energy; [7] n = 28. [8] Energy corrected milk, kg/Dry matter intake, kg; [9] N milk/N intake; n = 28; [10] Measured using a GreenFeed unit incorporated in the AMS; number of visits per measurement week (mean ± SD): 12 ± 7.1, 15 ± 8.0, and 11 ± 8.4 for cows on Maize50, Grass, and Maize50 treatments, respectively; n = 24.

Table 4. Effects of partial replacement of grass silage with maize silage on feed intake, diet digestibility, lactation performance, and gas emissions in experiment 3

	Treatments[1]				Probability	
	Maize50	Grass	Maize50	SEM[2]	Linear (Time)	Quadratic (Forage)
Intake, kg d^{-1}						
Dry matter	27.3	25.9	27.3	1.06	0.97	0.001
NDF[3]	9.15	9.32	9.32	0.395	0.28	0.49
Crude protein	4.23	4.69	4.28	0.161	0.44	< 0.001
Starch	6.01	4.12	6.00	0.222	0.90	< 0.001
MP[4]	2.58	2.48	2.57	0.094	0.72	0.01
PBV[5], g	379	967	457	24.6	0.01	< 0.001
ME[6], MJ d^{-1}	288	274	286	10.2	0.66	0.002
Digestibility, g kg^{-1}						
Dry matter	702	746	675	5.3	< 0.001	< 0.001
NDF	474	632	469	12.1	0.76	< 0.001
Crude protein	668	731	633	7.6	< 0.001	< 0.001
Starch	992	991	990	0.9	0.01	0.88
Milk production, kg d^{-1}						
Milk	43.7	42.1	42.2	2.49	0.02	0.11
ECM	45.5	46.1	45.7	2.23	0.83	0.51
Milk composition, g kg^{-1}						
Fat	42.3	46.2	45.0	1.54	0.02	0.01
Protein	37.5	37.7	38.2	1.31	0.35	0.79
Lactose	46.0	46.3	46.2	0.30	0.52	0.48
Urea, mg dl^{-1}	20.7	34.4	22.2	1.09	0.08	< 0.001
Feed utilization						
Feed efficiency[7]	1.67	1.78	1.67	0.051	0.93	< 0.001
N utilization[8]	0.382	0.333	0.372	0.0091	0.22	< 0.001
Gas emissions[9]						
CH_4, g d^{-1}	495	531	496	29.0	0.92	0.02
CH_4, g kg^{-1} DMI	18.2	20.7	18.3	0.97	0.75	< 0.001
CH_4, g kg^{-1} ECM	11.0	11.7	11.1	0.74	0.87	0.04
CO_2, g d^{-1}	13559	13886	14096	475.0	0.08	0.81
H_2, g d^{-1}	1.09	1.41	1.56	0.167	0.001	0.39

[1] Forages in different proportions: Grass = 100% grass silage; Maize50 = 50% of grass silage DM replaced by maize silage; [2] Largest SEM reported; n = 29 unless otherwise stated. Data are presented as least square means; [3] NDF = neutral detergent fibre;[4] MP = Metabolizable protein; [5] PBV = Protein balance in the rumen; [6] ME = Metabolizable energy; [7] Energy corrected milk, kg/Dry matter intake, kg. [8] N milk/N intake; [9] Measured using a GreenFeed unit incorporated in the AMS; number of visits per measurement week (mean ± SD): 18 ± 4.3, 19 ± 2.5, and 17 ± 4.3 for cows on Maize50, Grass, and Maize50 treatments, respectively; n = 28

Rumen fermentation

Partial replacement of grass silage with maize silage had no significant effect on rumen pH and total VFA concentration in EXP1 (Table 5). However, changes in molar proportions of VFA indicated a shift in the fermentation pattern. Increasing the proportion of maize silage in the diet decreased linearly ($p < 0.001$) the molar proportions of acetate, isobutyrate, and isovalerate, while it increased linearly ($p \leq 0.01$) the proportions of propionate and butyrate. The concentration of NH_3-N decreased linearly with increasing proportion of maize silage in the diet. We observed significant interactions ($p < 0.05$) between treatment and time for molar proportions of several VFA and concentration of NH_3-N. The difference in the proportions of acetate and propionate between treatments was greater before morning feeding and again in late afternoon, while the molar proportion curves tended to converge at 6 h sampling time (Supplemental Figures 1 and 2). In contrast, the difference between NH_3-N concentrations of grass and maize treatments was largest at 6 h sampling time and smaller before morning feeding and in late afternoon (Supplemental Figure 3).

Methane emissions

Adding maize silage to the diet had no significant effect on CH_4 emissions in EXP2 (Table 3), while we observed a 6.7% decrease in EXP3 (quadratic effect, *p* = 0.03) (Table 4). Maize silage also decreased CH_4 yield in EXP2 and EXP3, and intensity in EXP3 (quadratic effects, *p* = 0.02, *p* < 0.001 and *p* = 0.04, respectively). There were no diet-related differences in hydrogen emissions.

Table 5. Effects of partial replacement of grass silage with maize silage on rumen fermentation in experiment 1

	Treatments[1]				Probability	
	Grass	Maize25	Maize50	SEM[2]	Linear	Quadratic
pH	6.33	6.29	6.27	0.041	0.11	0.76
NH3-N, mmol l^{-1} [3]	8.27	5.45	3.94	0.523	<0.001	0.31
Total VFA[4], mmol l^{-1}	111	107	108	2.0	0.20	0.25
Molar proportions, mol %						
Acetate[3]	62.9	61.9	60.3	0.48	<0.001	0.33
Propionate[3]	20.9	21.5	22.5	0.22	<0.001	0.54
Butyrate	11.7	12.2	13.0	0.54	0.01	0.72
Isobutyrate[3]	0.91	0.85	0.80	0.023	<0.001	0.91
Valerate[3]	1.55	1.53	1.58	0.028	0.33	0.18
Isovalerate[3]	0.60	0.55	0.53	0.015	<0.001	0.33
Caproate	1.46	1.36	1.34	0.045	0.09	0.45

[1] Forages in different proportions: Grass = 100% grass silage; Maize25 = 25% of grass silage DM replaced by maize silage; Maize50 = 50% of grass silage DM replaced by maize silage; [2] n = 144 in the repeated measurement analysis; [3] Significant ($p < 0.05$) treatment × time interaction. [4] VFA = Volatile fatty acids.

Discussion

Short growing season, low temperatures and early frost may substantially affect forage maize maturation and composition at northern latitudes. The current study aimed to investigate, how the maturity of forage maize and potentially suboptimal composition of maize silage affects feed and nutrient intake, milk production performance, rumen fermentation, and CH_4 emissions in dairy cows.

Feed composition, intake, and digestibility

In northern conditions, the growing season is often too short for maize to mature, even when mulch film is used, as in this study. Lehtilä et al. (2023) reported that although the use of mulch film increased forage maize DM yield in Northern European climate conditions, the effect on DM and starch content was inconsistent between years. For EXP1 and EXP2, the forage maize was harvested as late as possible, and the DM content of the silage used in the experiments was at the upper limit of the optimal range of 300–350 g kg^{-1} reported by Khan et al. (2015). Despite achieving a high DM content through late harvest, the starch content remained below the mean value (301 g kg^{-1} DM) reported for wet (250–290 g DM kg^{-1}) maize silage by Khan et al. (2015). The forage maize used in EXP3 was harvested at the earlier state of maturation, resulting in lower DM and starch contents compared with EXP1 and EXP2. This was in line with the lower ETS in EXP3 due to the shorter growing period. Yet starch contents of the maize silages used in all three experiments were much greater than those (44–79 g kg^{-1} DM) reported by Mussadiq et al. (2012) from a comparable latitude in Sweden, and they were equal to or greater than those observed by Seleiman et al. (2017) in Southern Finland. In Finnish conditions, the DM and starch contents of maize silage are often much lower than those observed in this study and there is considerable annual variation (Seleiman et al. 2017, Liimatainen et al. 2022). Annual variation continues to be a problem at northern latitudes, despite climate warming. Based on a simulation study, Eckersten et al. (2012) estimated that by the end of the 21st century, a high forage maize quality would not be achieved in about 30% of the years in the middle of Sweden (60° N). Recently, a study by Lehtilä et al. (2023) showed that ETS of 670–710 °Cd was insufficient to increase starch content above 100 g kg^{-1} DM in Central Finland.

The average increase in DMI when 50% of grass silage was replaced with maize silage was 1.3 kg DM d^{-1}, which is close to the average increase of 1.55 kg DM d^{-1} reported in the summary by Khan et al. (2015). The positive feed intake response was achieved despite a substantial decrease in DM digestibility, and especially that of NDF. The greater DMI may be due to the faster rumen digestion and fermentation of maize silage owing to the greater starch content and smaller particle size (Dewhurst 2013, Khan et al. 2015). Consequently, rumen distension is decreased enabling greater DMI. Positive DMI response was also observed in EXP3, indicating that a relatively small difference in starch content between different maturities (EXP1 and EXP2 vs. EXP3) had no major effect on the DMI response of maize silage. Phipps et al. (2000) observed a large increase of DMI (+2.4 kg d^{-1}) between maize silages harvested from very early and early maturity crops (starch concentrations 114 vs. 274 g kg^{-1} DM). However, they also reported that the very early maturity (<250 g kg^{-1} DM) maize silage increased DMI compared with high-quality grass silage. Similarly, O'Mara et al. (1998) observed that DMI was markedly increased when grass silage was partly replaced by very early maturity (starch concentration 219 g kg^{-1} DM) maize silage.

In the current study, the relatively high DMI response of maize silage inclusion was contributed by the moderate digestibility of grass silages (D-value < 660 g kg^{-1} DM). Keady et al. (2008) reported greater DMI response (+2.25 vs. +0.51 kg d^{-1}) when low-ME (9.8 MJ kg^{-1} DM) rather than high-ME (12.0 MJ kg^{-1} DM) grass silage was replaced with 40% of maize silage. In a study by Sairanen and Kajava (2020), replacing early-harvested, high-digestibility grass silage (D-value 713 g kg^{-1} DM) partially with maize silage decreased DMI, even though the maize silage had an exceptionally high starch content (268 g kg^{-1} DM) for being harvested at the high latitude (63 09° N).

The inclusion of maize silage in the diet decreased the digestibility of DM and particularly the NDF digestibility. The decrease in NDF digestibility can be attributed to the low ruminal digestibility of potentially degradable NDF of maize silage (Brask et al. 2013). There were no systematic differences in indigestible NDF contents between maize and grass silages. The lack of differences in rumen pH in EXP1 suggests that rumen pH was probably not a major factor contributing to the differences in digestibility, in agreement with Brask et al. (2013). Starch accumulation in the kernels improves the digestibility of maize silage because starch is nearly completely digested in the digestive tract (Khan et al. 2015). On the other hand, increasing stem NDF content and decreasing NDF digestibility during the growing season reduce the digestibility of maize silage. As the starch content increases throughout the growing season, the NDF content decreases, compensating for the poor NDF digestibility of maize silage (Khan et al. 2015). The starch/NDF ratio was lower (0.56) in EXP3 than in EXP1 and EXP2 (0.70 and 0.65) reflecting the earlier maturity stage. Therefore, the negative effects of maize silage on the digestibility of the diet can be accentuated if forage maize is harvested at a very early maturity stage.

Milk production and composition

In EXP1, the observed milk yield response of 1.2 kg day^{-1} at a 50% inclusion rate of maize silage was lower than the average response of 1.9 kg day^{-1} found in the summary by Khan et al. (2015). The observed response was modest, considering the relatively low digestibility and ME content of grass silage. In EXP2 and EXP3, no milk yield response was observed despite increased DMI. Further, milk protein composition or yield was not affected in any of the experiments, contradicting the review by Khan et al. (2015). However, it should be noted that the experimental design of EXP2 and EXP3 may have limited the ability to detect statistically significant differences, since the effects of forage and period could not be separated in the analysis.

The increased milk yield in EXP1 was primarily due to greater ME intake induced by the replacement of grass silage with maize silage, while feed conversion to milk yield was not affected. The lack of milk yield response in EXP2 and EXP3 was probably due to the lower starch/NDF ratio of maize silage compared to EXP1. Khan et al. (2015) observed that milk yield was strongly positively related to starch/NDF ratio during maturation of the maize. The milk yield response achieved by adding maize silage to the diet depends significantly on the type of grass silage that is replaced by maize silage. In the study by Sairanen and Kajava (2020), partial replacement of high-digestibility grass silage with maize silage (26% DM) did not affect milk yield. Another factor contributing the variation of observed responses between experiments is balancing nutritive differences of forages by concentrate feeding. In some studies, these differences have been partly or fully balanced (Brask-Pedersen et al. 2023), whereas in other studies forage source is changed without changing concentrate proportion or composition in the diet (van Gastelen et al. 2015).

It should be noted that the diets were not isonitrogenous and thus the inclusion of maize silage in the diet reduced dietary CP content. In EXP1 and EXP2 the reduction of dietary CP content was 14 g kg^{-1} DM, whereas it was considerably larger (25 g kg^{-1} DM) in EXP3, owing to the greater CP content of grass silage. Because of its lower crude protein content, replacing grass silage with maize silage consistently reduced milk urea concentration across all experiments.

Rumen NH_3-N concentration decreased linearly in EXP1 with the increasing proportion of maize silage in the diet. The milk protein yield was, however, unaffected by treatments in EXP1, suggesting that differences in dietary CP concentrations between forages were probably not a limiting factor for microbial protein synthesis in this study. Further, the rumen NH_3-N concentration of Maize50 diet was well above the concentration (4.1 mg 100 ml^{-1} / 2.9 mmol l^{-1}) needed to maximize bacterial protein synthesis according to Russell and Strobel (1987). Ahvenjärvi and Huhtanen (2018) reported that rumen microbes efficiently captured ammonia-N from rumen fluid when rumen ammonia-N concentrations remained low (3.5 mg 100 ml^{-1}), indicating that rumen ammonia-N concentration was an insensitive indicator of N deficiency at low levels of diet CP. On the other hand, an increased supply of rumen fermentable carbohydrates with maize silage in the form of starch enhances microbial protein production (Givens and Rulquin 2004, Brask-Pedersen et al. 2023) and compensates lower CP content of the feed. This was reflected in the increase of estimated MP intake with maize silage diets. Further, the dietary CP concentration (150 g kg^{-1} DM) of Maize50 was above the suggested inflection point for rumen ammonia-N accumulation at dietary CP 147 g kg^{-1} DM (Broderick et al. 2010). Therefore, rumen degradable CP supply was most likely sufficient at a 50% inclusion level of maize silage, as also suggested by the positive protein balance value in the rumen. Nevertheless, in conjunction with reduced milk urea concentration, partial replacement of maize silage with grass silage improved nitrogen utilization, in line with earlier studies (Burke et al. 2007, van Gastelen et al. 2015).

Methane emissions

The inclusion of maize silage decreased CH_4 emissions (–6.7%), yield (–11.8%), and intensity (–5.6%) in EXP3. A decreased yield (–7.3%) and a numerical tendency ($p = 0.11$) towards a decrease in CH_4 emissions (–4.2%) was also observed in EXP2. The decrease of CH_4 emissions with maize silage inclusion can be attributed to differences in starch and NDF content, and lower ruminal fiber digestibility with maize silage diets, inducing decreased acetate to propionate ratio in the rumen (Brask et al. 2013, Hammond et al. 2016, Vanhatalo and Halmemies-Beauchet-Filleau 2020). The slightly smaller reduction in CH_4 emissions and yield observed in EXP2 compared to EXP3 is inconsistent with the observed differences in starch content of maize silage. However, lower number of acceptable visits by the cows selected for sampling in EXP2, along with the need to supplement data using additional cows may have introduced uncertainty in the interpretation.

In line with current results, Doreau et al. (2014) observed lower CH_4 emissions (–11.6%), yield (–10.3%), and intensity (–12.8%) with diets containing 45% forage as maize silage rather than grass silage. Van Gastelen et al. (2022) reported decreased CH_4 emissions (–6.5% and –8.9%), yield (–10.5% and –17.5%), and intensity (–7.1% and –15.0%) with increasing proportion of maize silage (0%, 28%, or 56%) in the diet. Van Gastelen et al. (2015) observed that the effect of replacement of grass silage with maize silage on CH_4 emissions was curvilinear, and they suggested that critical dietary concentration of starch is required to decrease CH_4 emissions. Accordingly, Hatew et al. (2016) concluded that increasing maturity of maize and consequently increasing starch content (between 275 and 385 g kg^{-1} DM) at harvest reduced CH_4 emissions linearly. At northern latitudes high enough starch content of maize silage to substantially reduce enteric CH_4 emissions is most likely not achieved in all years.

Conclusions

We observed that partial replacement of grass silage with maize silage of relatively high dry matter and starch content for northern latitudes increased feed intake, decreased digestibility of the diet, and improved nitrogen utilization, while the effects on milk production were less consistent. Despite increased feed intake, milk production responses were low or even lacking, in comparison with moderate-digestibility grass silages. Harvesting forage maize at early maturity due to the short growing season may diminish or nullify the positive effects of maize silage relative to grass silage, because early maturity limits starch accumulation in kernels and thus decreases nutritive value of maize silage described by starch/NDF ratio. Partial replacement of grass silage with maize silage reduced enteric CH_4 yield by an average of 9.5% and total emissions by 5.5%, whereas a reduction in emission intensity (5.6%) was observed in only one experiment. Provided that sufficient maturity is achieved, partial replacement of grass silage with maize silage may be a useful option for improving production performance and mitigating enteric CH_4 emissions in northern conditions. However, annual variation in growing conditions and nutritive quality introduces uncertainty in production responses, which may reduce interest in cultivating forage maize.

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Appendix

Supplemental Table 1. Dates of the first night frost, monthly mean temperatures and monthly precipitation sums during the growing seasons of 2019 and 2020, and the long-term average (1991–2020) in Kaisaniemi Helsinki (60° 10’ 17.40” N, 24° 56’ 26.99” E)

		2019	2020	1991–2020
First night frost, date		8 Oct	5 Oct	11 Nov
Temperature, °C	May	10.3	9.6	10.4
	June	17.3	17.9	14.9
	July	17.5	16.7	18.1
	August	17.3	17.1	16.9
	September	12.2	13.8	12.3
	October	6.2	9.3	6.6
Precipitation, mm	May	62	53	38
	June	16	75	60
	July	73	83	57
	August	82	77	81
	September	77	55	56
	October	102	64	73

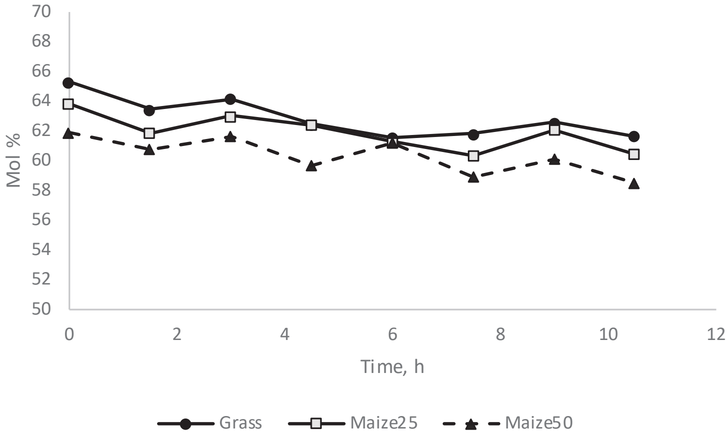
Supplemental Table 2. Fermentation quality of maize and grass silages (g kg^{-1} DM)

	Exp. 1		Exp. 2		Exp. 3	
Item	Maize	Grass	Maize	Grass	Maize	Grass
pH	4.37	4.43	4.40	4.39	4.40	4.39
Water-soluble carbohydrates	74.7	61.4	108	109	100	24.2
Lactic acid	30.0	41.1	27.8	25.1	31.1	92.3
Acetic acid	13.1	10.7	13.8	6.10	8.63	14.6
Propionic acid	0.610	1.64	0.700	0.970	2.21	0
Butyric acid	0	0.380	0	0	0	0.830
Ethanol	3.38	2.39	1.74	2.34	4.64	5.34
NH_3-N, g kg^{-1} N	86.8	77.9	90.0	49.9	41.4	78.3

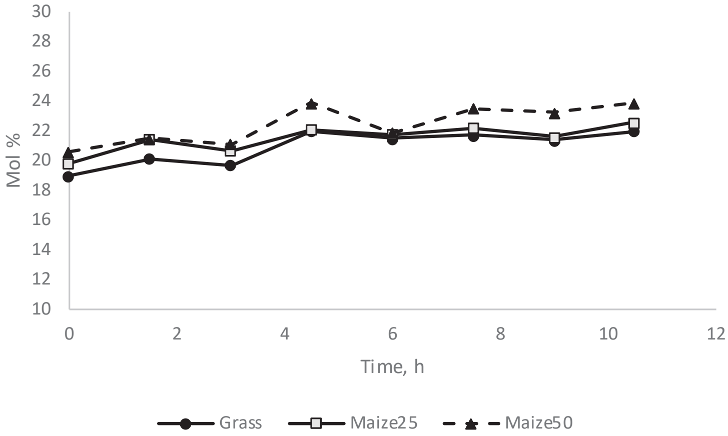
Supplemental Table 3. Chemical composition of concentrates

	Exp. 1		Exp. 2		Exp. 3	
Item	Conc. mix[1]	Comp. feed[2]	Conc. mix[1]	Comp. feed[2]	Conc. mix[3]	Comp. feed[2]
Dry matter, g kg^{-1}	864	873	868	867	867	870
Chemical composition (g kg^{-1} DM)						
Ash	56.4	73.4	74.0	72.9	58.4	79.5
Crude protein	173	222	174	223	167	230
Total fat	24.6	44.1	26.2	44.1	26.5	52.2
NDF[4]	159	224	149	221	184	229
NFC[5]	588	436	578	439	564	409
Starch	411	210	415	205	425	190
MP	97.9	124[6]	97.9	124[6]	97.4	124[6]
PBV[7]	11.9	53.0[6]	11.9	53.0[6]	10.8	56.0[6]
ME, MJ kg^{-1} DM	12.6	12.6[6]	12.6	12.6[6]	12.6	12.4[6]

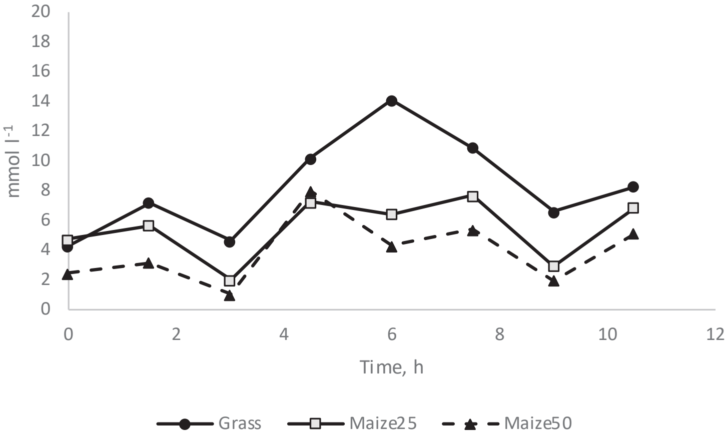
[1] Concentrate mix consisted of (% in DM) barley (51%), oats (15%), faba beans (20%), peas (5%), molassed sugar beet pulp (5%), minerals and vitamins (3%), and propylene glycol (1%); [2] Compound feed contained (% in DM) rapeseed meal (42%), oats (15%), barley (15%), and molasses (8%) as major ingredients (Hankkija Oy, Hyvinkää, Finland); [3] Concentrate mix consisted of (% in DM) barley (52%), oats (17%), faba beans (22%), molassed sugar beet pulp (5%), minerals and vitamins (3%), and propylene glycol (1%); [4] NDF = Neutral detergent fibre; [5] NFC = Non-fibre carbohydrates; [6] Values provided by the manufacturer; [7] PBV = Protein balance value in the rumen



Supplemental Figure 1. Effects of partial replacement of grass silage with maize silage on the molar proportion of acetate in the rumen (experiment 1). Rumen fluid samples were collected at 1.5 h intervals starting before morning feeding at 0530 h. Data were analyzed using a linear mixed model with repeated measures ANOVA (n = 144). Significant ($p < 0.05$) treatment × time interaction.



Supplemental Figure 2. Effects of partial replacement of grass silage with maize silage on the molar proportion of propionate in the rumen (experiment 1). Rumen fluid samples were collected at 1.5 h intervals starting before morning feeding at 0530 h. Data were analyzed using a linear mixed model with repeated measures ANOVA (n = 144). Significant ($p < 0.05$) treatment × time interaction.



Supplemental Figure 3. Effects of partial replacement of grass silage with maize silage on rumen NH3-N concentration (experiment 1). Rumen fluid samples were collected at 1.5 h intervals starting before morning feeding at 0530 h. Data were analyzed using a linear mixed model with repeated measures ANOVA (n = 144). Significant ($p < 0.05$) treatment × time interaction.