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Enteric and manure emissions from Holstein-Friesian dairy cattle fed grass silage–based or corn silage–based diets

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ABSTRACT

This study aimed to evaluate trade-offs between enteric and manure CH_4 emissions, and the size of synergistic effects for CH_4 and nitrogenous emissions (NH_3 and N_2O). Sixty-four Holstein-Friesian cows were blocked in groups of 4 based on parity, lactation stage, and milk yield. Cows within a block were randomly allocated to a dietary sequence in a crossover design with a grass silage-based diet (GS) and a corn silage-based diet (CS). The GS diet consisted of 50% grass silage and 50% concentrate, and CS consisted of 10% grass silage, 40% corn silage, and 50% concentrate (dry matter basis). The composition of the concentrate was identical for both diets. Cows were housed in groups of 16 animals, in 4 mechanically ventilated barn units for independent emission measurement. Treatment periods were composed of a 2-wk adaptation period followed by a 5-wk measurement period, 1 wk of which was without cows to allow separation of enteric and manure emissions. In each barn unit, ventilation rates and concentrations of CH_4, CO_2, NH_3, and N_2O in incoming and outgoing air were measured. Cow excretion of organic matter was higher for CS compared with GS. Enteric CH_4 and cow-associated NH_3 and N_2O emissions (i.e., manure emissions excluded) were lower for CS compared with GS (−11, −40, and −45%, respectively). The CH_4 and N_2O emissions from stored manure (i.e., in absence of cows) were not affected by diet, whereas that of NH_3 emission tended to be lower for CS compared with GS. In conclusion, there was no trade-off between enteric and manure CH_4 emissions, and there were synergistic effects for CH_4 and nitrogenous emissions when grass silage was exchanged for corn silage, without balancing the diets for crude protein content, in this short-term study.

Key words: dairy cow, methane emission, ammonia emission, nitrous oxide emission

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INTRODUCTION

The livestock sector is estimated to be responsible for approximately 14.5% of total global anthropogenic greenhouse gas (**GHG**) emissions (Gerber et al., 2013). Methane (CH_4) emission from enteric fermentation is the main source of GHG emissions from dairy cattle, representing 46.5% of the total GHG emissions in the dairy supply chain, whereas CH_4 emission from manure management represent only 3.8% of the total GHG emission in the dairy supply chain (Gerber et al., 2013). Many efforts have been made to mitigate enteric CH_4 emissions from dairy cows, including dietary strategies [reviewed by e.g., Hristov et al. (2013) and Arndt et al. (2022)]. However, manure CH_4 emissions are often overlooked when evaluating the CH_4 mitigation potential of dietary strategies, despite its potential importance. Forages contribute most to CH_4 emission (Johnson and Johnson, 1995), and hence the choice of forage type as well as the quality of the forage are important potential strategies to mitigate enteric CH_4 emissions (van Gastelen et al., 2019). Several studies demonstrated that an increased amount of corn silage at the expense of grass silage is an effective strategy to mitigate enteric CH_4 emissions in dairy cattle, without having negative effects on feed intake and lactation performance (e.g., Brask et al., 2013; Doreau et al., 2014; van Gastelen et al., 2015). Due to the discrepancies in literature, it remains unclear if and how CH_4 emission from manure is affected when feeding a corn silage-based diet. Furthermore, enteric and manure emissions were not measured simultaneously before and under practical housing conditions with presence of cows. Klevenhusen et al. (2011) reported a significant lower manure CH_4 emission from corn silage fed animals compared with hay fed animals, but only at 7 wk of storage at 14°C (not in any other combination of incubation duration and temperature). Hellwing et al. (2014) concluded that the reduced enteric CH_4 emissions for a corn silage-based relative to a grass silage-based diet, might be counteracted or complemented by increased CH_4 emissions from slurry storage (measured in 128-d in vitro incubations at 10, 15, and 20°C). Ramin et al. (2021) reported no effect of partially replacing grass silage with corn silage on CH_4 emission from feces (measured in 9-wk in vitro incubations of feces at 39°C), despite a larger OM and NDF output, but did not measure enteric CH_4 emissions. To qualify this dietary strategy (i.e., replacing grass silage with corn silage) as an overall effective CH_4 mitigation strategy for the dairy industry, the effect of the dietary strategy on both enteric and manure-derived methanogenesis needs to be explored simultaneously in vivo.

Next to CH_4 emissions, dairy production also contributes to environmental pollution from fecal and urinary N in the form of nitrogenous emissions (Dijkstra et al., 2018). Emissions of N_2O contribute to global warming (IPCC, 2021), and emissions of NH_3 can redeposit, causing N enrichment of sensitive ecosystems and subsequently eutrophication and acidification (Selbie et al., 2015). Emissions of N_2O are mostly the consequence of microbial decomposition of N compounds in the excreta, with urinary N being more susceptible than fecal N (Selbie et al., 2015). Emissions of NH_3 originate mainly from chemical and physical processes on the barn floor and in top layer of stored manure with urinary N as the main source (Sommer et al., 2006). As excessive feed N is mainly excreted with urine, a reduction of the dietary protein content will generally result in a reduction of the urinary N excretion (Dijkstra et al., 2013; Powell and Rotz, 2015) and subsequently in a reduction of nitrogenous emissions (e.g., Külling et al., 2001; Misselbrook et al., 2005). Feeding a low-protein, high-energy feed, such as corn silage, is one of the proposed dietary strategies that may reduce N excretion and subsequently nitrogenous emissions (e.g., van Gastelen et al., 2015; Dijkstra et al., 2018). It is at present unclear how the effects in CH_4 emissions upon replacing grass silage with corn silage may cause a trade-off or have a synergistic effect toward nitrogenous emissions, because studies reporting these gaseous emission sources simultaneously are limited.

The aim of this study was 2-fold. First, to evaluate the potential trade-off between enteric and manure CH_4 emissions of Holstein-Friesian dairy cows when replacing a grass silage–based diet for a corn silage–based diet on a DM basis under practical housing and manure storage conditions. Second, to evaluate to what extent these effects in CH_4 emissions are accompanied by a synergistic effect toward nitrogenous emissions. It was hypothesized that replacing grass silage with corn silage would result in decreased enteric CH_4 emissions and in increased fecal output of starch and NDF and subsequently manure CH_4 emissions. The increase in manure CH_4 emissions was not expected to trade-off the decrease in enteric CH_4 emissions. Additionally, we hypothesized that replacing grass silage with corn silage would result in reduced nitrogenous emissions (NH_3 specifically) as a synergistic effect.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted from October 2019 until January 2020 at the research facilities of Dairy Campus (Wageningen Livestock Research, Leeuwarden, the Netherlands), under the Dutch law on Animal Experiments in accordance with European Union Directive 2010/63. The experiment consisted of 2 dietary treatments, 4 barn units (mimicking regular cow housing, floor, and manure storage conditions) and 64 Holstein-Friesian dairy cows (19% first lactation, 33% second lactation, and 48% third lactation or higher) with an average milk production of 31.6 ± 8.09 kg/d (mean ± SD) and 160 ± 81 DIM at the start of the experiment. The cows were blocked in groups of 4. We first blocked animals by parity (priority, distinguishing between first, second, and third parity or higher). Then we subsequently blocked on milk production and DIM (to have animals in a similar metabolic condition and persistency of milk production). Within each block cows were randomly assigned to a dietary treatment sequence in a crossover design with 2 treatments, involving 2 replications and 2 periods. Dietary treatments were a grass silage-based diet (**GS**) and a corn silage-based diet (**CS**). Half of the cows received GS in period 1, and CS in period 2, whereas the other half received the dietary treatments in the opposite sequence. Each group of 16 cows was housed in 1 of 4 mechanically ventilated barn units (i.e., barn units A, B, C, and D; Figure 1) equipped for independent emission measurements. The experiment lasted 14 wk in total and was composed of 2 treatment periods, where the first treatment period was directly followed by the second treatment period. Both treatment periods lasted 7 wk and were composed of a 2-wk adaptation period followed directly by a 5-wk measurement period. The measurement period was composed of 4 wk with cows present in the barn units to measure the total emissions of CH_4, CO_2, NH_3, and N_2O, and 1 wk without cows in the barn units to measure CH_4, CO_2, NH_3, and N_2O emission from manure storage only (Figure 1). Due to limited housing capacity elsewhere at our research facility, we were not able to have the 4 barn units empty (i.e., without cows) at the same moment. Hence, we emptied the barn units on a weekly basis in a staggered approach (Figure 1), during which the cows were housed in another barn.

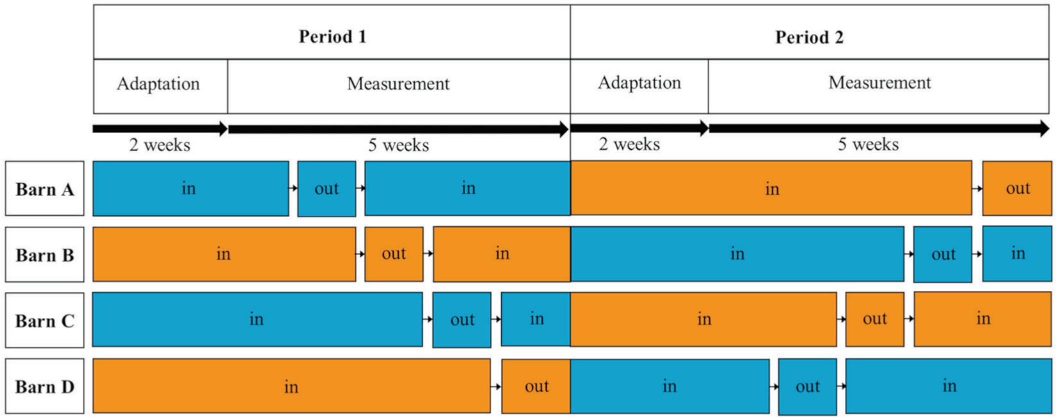

Figure 1. The experiment followed a crossover design with 2 periods and 2 dietary treatments: a grass silage-based diet (GS; blue) and a corn silage-based diet (CS; orange). Each period consisted of 2 wk of adaptation followed by 5 wk of measurement, where the measurement period was composed of 4 wk with cows present in the barn (in) and 1 wk without cows in the barn (out). At the start of each period, the manure pits were emptied and subsequently filled up again to 50 cm in height with a homogenized manure. The 64 dairy cows were housed in 4 mechanically ventilated units (barns A, B, C, and D; 16 cows per barn), and the weeks with empty barns followed each other in a staggered approach.

Diets and Feeding

Both diets consistent of 50% forage and 50% concentrates, on a DM basis. The composition of the concentrate was identical for both diets. The forage was 100% grass silage (i.e., perennial ryegrass) for GS, and 20% grass silage and 80% corn silage for CS (ingredient as percentage of total amount of forage in the diet, DM basis). The chemical composition of the individual ration ingredients is presented in Table 1. The ingredients and chemical composition of the dietary treatments are presented in Table 2. The concentrate was in meal form and produced by Agrifirm (Apeldoorn, the Netherlands) in one batch and hence of uniform composition

Table 1. Chemical composition (g/kg DM, unless stated otherwise) of the individual ration ingredients grass silage, corn silage, and concentrates

Item	Grass silage[1]	Corn silage[2]	Concentrate[3]
DM (g/kg)	391	350	911
OM	889	962	911
CP	179	87	165
Crude fat	35	42	48
Gross energy (MJ/kg DM)	19.0	19.2	18.2
NDF	394	409	339
ADF	242	240	211
ADL	1.7	14.4	40.5
Starch	ND	296	180
Sugar	122	ND	79
NE_L[4] (MJ/kg DM)	6.6	6.5	6.7
DVE[5]	59	48	92
OEB[6]	28	−31	0

[1]Ensiling characteristics: acetic acid = 16 g/kg DM, lactic acid = 68 g/kg DM, ammonia-N = 7% total N, and pH = 4.3.

[2]Ensiling characteristics: acetic acid = 24 g/kg DM, lactic acid = 27 g/kg DM, ammonia-N = 13% total N, and pH = 4.3.

[3]Ingredient composition (g/kg DM): palm kernel flakes = 340, citrus pulp = 170, wheat semolina = 150, corn = 114, wheat = 90, rumen-protected rapeseed meal (MervoBest, Nuscience) = 35, barley = 25, citrocol = 20, NaCl = 18, $CaCO_3$ = 16, sunflower seed meal = 10, urea = 5, MgO = 5, and trace mineral and vitamin premix = 2 (i.e., vitamin A, 5,015 IU; vitamin D_3, 1,212 IU; vitamin E, 25 mg; manganese, 112.8 mg; zinc, 27.3 mg; copper, 21 mg; iodine, 1.1 mg; selenium, 0.394 mg; iron, 306.3 mg; cobalt, 0.304 mg; molybdenum, 0.100 mg).

[4]van Es, 1978.

[5]Intestinal digestible protein (van Duinkerken et al., 2011).

[6]Rumen-degraded protein balance (van Duinkerken et al., 2011).

Table 2. Ingredient and chemical composition (g/kg DM, unless stated otherwise) of the grass silage-based diet (GS) and the corn silage-based diet (CS)

Composition	GS	CS
Ingredient		
Grass silage	500	100
Corn silage	0	400
Concentrate	500	500
Chemical composition		
DM (g/kg)	421	403
OM	900	929
CP	172	135
Crude fat	41	44
Gross energy (MJ/kg DM)	18.6	18.7
NDF	366	373
ADF	227	226
ADL	21	26
Starch	90	208
Sugar	101	52
NE_L (MJ/kg DM)[1]	6.7	6.6
DVE[2]	76	71
OEB[3]	14	−10

[1]Net energy for lactation (van Es, 1978).
[2]Intestinal digestible protein (van Duinkerken et al., 2011).
[3]Rumen-degraded protein balance (van Duinkerken et al., 2011).

throughout the entire experiment. The grass silage and corn silage were from the same batch throughout the entire experiment, where the chop length at harvest was 15 and 7 mm, respectively.

The different dietary components were stored in bunkers (used for forages) and a silo (used for the concentrate). On a weekly basis (based on the DM content from the previous week of each individual dietary component, measured daily), the required quantity of each of these components was determined to prepare the diets. Daily, the grass silage, corn silage (only CS), concentrate, and water were mixed to compose a TMR using a self-propelled mixer wagon Triotrac (model JCB/FPT Tier 4/Fase IV; Trioliet, Oldenzaal, the Netherlands) equipped with a cutter loader system and an electronic weighing scale. Water was added to the TMR to achieve the target DM content of 400 g/kg to enhance feed intake. The TMR was fed immediately after preparation. The diets were offered once daily at 0800h at the feeding fence of each barn unit (i.e., feeding at group level; 16 cows per barn unit) and pushed up by a feed pushing robot (Moov pro 2.0, JOZ B.V., Westwoude, the Netherlands) 8 times daily (0100, 0330, 1300, 1400, 1500, 1700, 1900, and 2100 h). The daily amount of TMR consumed by the groups of cows was calculated from the amounts of feed fed in the morning and the refusals present at the feeding fence before the morning feeding. Cows were fed ad libitum, allowing at least 10% refusals. Cows had free access to clean drinking water throughout the experiment.

Housing

The dairy cows were housed in 4 mechanically ventilated barn units for measurement of gaseous emissions (i.e., barn units A, B, C, and D; 16 cows per barn unit). Each barn unit was equipped with 16 cubicles, 28 feeding places at the feeding fence, 1 drinking place, and 1 automatic cow brush. The walls of the ventilated barn units were constructed to minimize air transport apart from the mechanical ventilation points and the front of the feeding fence. With a large curtain the front of the barn unit can be closed to regulate the inlet of air and allow accurate measurements of gaseous emissions. Each barn unit consisted of a slatted floor area and 1 row of 16 cubicles. The slatted floor was scraped every 2 h. Slurry was stored underneath the slatted floor and underneath the cubicles. The total slatted floor area in each unit was 83 m^2 (5.2 m^2 per animal). The slot width was 35 mm and the beam width was 122 mm. Total tread area was approximately 80%. The total surface area of the slurry storage was 143 m^2 divided in 2 channels of 1.4 m deep and 25 m long, one being 3.5 m wide and the other 2.2 m wide. Total slurry storing capacity was 200 m^3.

When measurements of manure emissions only took place (Figure 1), the cows of that particular barn unit were housed in a separate barn. This barn was equipped with 16 cubicles, 16 feeding places at the feeding fence, 1 drinking place, and 1 automatic cow brush. During this period, cows continued to receive their assigned diet and were fed similarly as when they were housed in the barn units for gas measurements (described above).

The dairy cows were milked twice daily between 0500 and 0600 h and 1500 to 1600 h throughout the experiment, both when housed in 1 of the 4 measurement barn units and when housed in the other barn. Cows were milked in a rotary milking parlor of 40 stands with automatic cow identification, milk recording, and milk cluster removal (AutoRotor PerFormer, Gea Farm Technologies, Leeuwarden, the Netherlands). The dairy cows received no concentrate feeding during milking.

Sampling and Measurements

Feed, Milk, and BW. Samples of grass silage, corn silage, and concentrate were taken once weekly and stored at −20°C pending analysis. Once weekly, for 1 morning and for 1 evening milking event, a milk sample (10 mL) from each cow was collected in a tube containing sodium azide (5 μL) for preservation, stored no longer than 1 d at 4°C, and analyzed for fat, protein, lactose, and urea content. A weighted average daily milk composition was calculated from milk composition and milk yield of both daily milking events. The BW of each cow (identified via a monitor ID system) was recorded twice daily during the entire study. Cows were weighed as they exited the milking parlor using a Gea weighing scale (Gea Farm Technologies).

Manure Height and Samples. At the start of both periods, the manure pits (for slurry storage) below the slatted floor in each barn unit was emptied. We subsequently filled the manure pits again with a homogenized manure (i.e., manure stored for more than 1 mo in another barn of the research facility Dairy Campus) to ensure the same manure composition for the different barn units at the start of the measurements (i.e., avoiding initial differences for dietary treatments; Figure 1). We targeted to fill the manure pits up to 50 cm in height (i.e., 69 m^3), and we realized on average 67 ± 3.9 m^3 overall, with 68 ± 2.1 m^3 for manure pits for GS and 66 ± 5.2 m^3 for the manure pits for CS. The homogenized manure originated from dairy cows that were fed according to practice (i.e., no dietary treatments). Manure samples were collected directly after filling the manure pits. At the end of both measurement periods, the slurry in the manure pits was mixed mechanically and subsequently sampled. The manure samples were stored at −20°C pending analysis. Throughout the trial, the height of the slurry in each manure pit was determined at 3 places once weekly to calculate manure production.

Emission Measurements. Each barn unit was ventilated by 2 axial ventilators with a diameter of 80 cm (1680M, Fancom B.V., Panningen, the Netherlands). The ventilation rate (m^3/h) of both ventilators in each barn unit was calculated from the pulse signal of a calibrated free running fan (ATM80, Fancom B.V.) mounted in the ventilation tube. The ventilators were kept at a constant rate by a controller (FC14, Fancom B.V.), with and without the cows being present in the emission barns. Ventilation rate was logged and stored with a datalogging system (CR1000X, Campbell Scientific Inc., Logan, UT). The average ventilation rate in cubic meters per hour was subsequently calculated per barn unit (i.e., based on 2 ventilators) and per hour (i.e., to come to a similar time frame as to that of the gas concentration measurements; see description below) using a relation between pulse signal and volumetric flow rate that have been previously determined in a wind tunnel study for each ventilator separately.

For each barn unit, air samples from the ingoing air were taken at the bottom of the curtain which was closed to regulate the air inlet (i.e., close the front of the barn unit with the feeding fence) and air samples from the outgoing air were taken from each ventilator both through sampling lines with a diameter of 0.635 cm (Figure 2). Individual sampling lines (12) of each ventilator (8) and background air sampling (4) were kept separated until the distribution manifold (A0311, Picarro Inc., Santa Clara, CA), which was placed within close distance (<30 cm) of the multigas analyzer for CH_4, CO_2, NH_3, and N_2O (G2508, Picarro Inc.). To prevent cross-contamination the common tube between distribution manifold and multigas analyzer was flushed for 4 min before taking a sample for 1 min. Sample flow and bypass flow was created by 2 diaphragm pumps (MD1, Vacuubrand, Wertheim, Germany) before switching to the next sampling line. The sampling lines always followed the same sequence within (i.e., inlet air, outgoing air 1, and outgoing air 2) and between (i.e., barn unit A, B, C, and D) barn units, which resulted in a sampling interval of 1 h for each sampling line and 15-min measurement for each barn unit within every hour. The Picarro system was calibrated at the start of the experiment in the Air Quality Laboratory of Wageningen Livestock Research, where CH_4, CO_2, NH_3, and N_2O were offered in different concentrations. A calibration line was obtained for each gas which was applied to the data collected during the present study. Furthermore, air temperature and relative humidity were measured at 2 spots in each barn unit and on 2 spots outside the barn units (HMP 60, Vaisala Oyj, Vantaa, Finland). Average air temperature and relative humidity values were stored every 15 min in the datalogging system.

Chemical Analyses. At the end of the experiment, the feed and manure samples were thawed at room temperature, freeze-dried until constant weight, and ground to pass a 1-mm screen by using a cross beater mill for both silages (Peppink 100AN, Olst, the Netherlands) and an ultracentrifugal mill for the concentrate and manure (Retsch ZM200, Retsch GmbH, Haan, Germany). The grass silage, corn silage, and concentrate samples were analyzed for DM, ash, total N, NH_3-N, crude fat, starch (corn silage and concentrate only), sugars (grass silage and concentrate only), NDF, ADF, ADL, and gross energy (**GE**). Manure samples were analyzed for DM, ash, total N, NH_3-N, crude fat, starch, NDF, ADF, ADL, and carbon. The analyses were performed as described by Nichols et al. (2018). Bomb calorimetry (ISO 9831; ISO, 1998) was used to determine GE. The CP content was calculated as N × 6.25. Milk samples were analyzed for fat, protein, lactose, and urea content by mid-infrared spectroscopy (Qlip B.V., Zutphen, the Netherlands; ISO 9622; ISO, 2013).

Calculations. Reported values for nutrient content of the diets (Table 2) were calculated from ration com-

Figure 2. Overview of 1 of the 4 barn units, with curtain in lifted position. Red line indicates the sampling line for incoming air concentrations. The yellow arrows indicate the 2 ventilators including sampling lines for outgoing air concentrations.

position and analyzed values obtained for the grass silage, corn silage, and concentrate (Table 1). The NE_L was calculated with the VEM (Dutch feed unit lactation) system according to van Es (1978). For both silages, digestible protein, rumen-degradable protein balance (see Table 1), and NE_L were calculated based on the chemical composition as obtained by near-infrared spectroscopy analysis (Eurofins Agro, Wageningen, the Netherlands). For the concentrate, digestible protein, rumen-degradable protein, and NE_L were calculated based on table values for composition of the ingredients (CVB, 2018).

Fat- and protein-corrected milk yield (**FPCM**) was calculated according to the equation FPCM (kg/d) = (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d) (CVB, 2018). Manure production was determined by discriminating between the amount of homogenized manure at the start and the amount of manure produced during the measurement periods (representing the dietary treatments). Together with analysis of the manure samples collected at the start as well as at the end of each measurement period, the excretion of nutrients was calculated.

Starch, NDF, and crude fat were assumed to be absent in urine, allowing for calculation of apparent total-tract digestibility (**ATTD**) of these components from analysis of starch, NDF, and crude fat in manure and feed. Per barn unit, we calculated the total intake of each of these nutrients as well as the total excretion of each of these nutrients, and subsequently subtracted the total excretion of each nutrient from the total intake of each nutrient. The outcome was assumed to be the fraction of nutrients that was digested, which we expressed as percentage of total intake.

Gas concentrations were measured in ppm and after application of the calibration line converted to mg/m^3 using molar volume at 20°C (i.e., fixed temperature, not adjusted for actual temperature in the barn units). Production of CH_4, CO_2, NH_3, and N_2O (g/h) was calculated from the difference between outgoing and incoming air gas concentration (in g/m^3), multiplied with the measured volumetric flow rate (in m^3/h). The gas concentration of the outlet air was represented by the hourly average of the measured gas concentration at the 2 ventilators within a barn unit.

During the weeks in which cows were not present in the barn units (i.e., representing manure emissions), we only used the data from d 3 onwards (i.e., d 3–7; 5 d in total). We excluded the measurements of d 1 and 2, because d 1 represented a transition day at which housing conditions were altered (taking cows out of the barn unit after morning milking; no full 24 h empty barn unit for measurements) and because we observed that the emissions of NH_3 reached a stable plateau, representing manure emissions, after d 2. We subsequently calculated a manure contribution factor per barn unit and per measurement period between the gas emissions measured when cows were absent and when the cows were present in the barn unit (i.e., using only the week directly before the week when the cows were absent). This factor was subsequently applied to all measurements of that particular barn unit and in all the weeks that cows were present (also after the week when cows were absent), to allow separation of enteric and the other cow presence associated emissions from emissions from the manure in the manure pit beneath the slatted floor. Based on the separation of enteric CH_4 emission from manure CH_4 emission from the manure, we calculated the fraction of enteric and manure CH_4 emission from the total daily CH_4 emission. For doing this, the total amount of manure, the number of days before the measurement without dairy cows in the barn unit, and the number of cows in the barn unit were taken into account to calculate the manure CH_4 emissions on an individual animal and daily basis, such as that of enteric CH_4 emission.

Enteric and manure CH_4 emissions were converted to CO_2 equivalents by assuming a global warming potential (**GWP**) of 27, whereas for cow-associated and manure emissions of N_2O, a GWP of 273 was assumed (IPCC, 2021). To convert cow-associated and manure emissions of NH_3 to CO_2 equivalents, we first calculated indirect N_2O emissions from NH_3 as described by Lagerwerf et al. (2019; 1% indirect N emissions with N_2O from total N emissions with NH_3) for which we subsequently assumed a GWP of 273 (IPCC, 2021).

Statistical Analysis

Only data from the measurement periods were used for data analyses; days on which a change in housing took place [e.g., days at which cows were taken out of the barn units (when emptying the barn units) or at which cows were placed back into the barn units (when filling the barn units again after being empty for 1 week, not representing a full 24-h period)] were excluded from the data set. The barn units were the experimental unit. Hence, all variables were averaged per week and per barn unit, including the variables that were measured on individual animal level (e.g., milk yield and composition). For animal related variables (e.g., feed intake, milk production and composition, and BW), only the data collected when the cows were housed in 1 of 4 barn units were used for analysis. To determine the manure emissions, only data collected when no cows were housed in 1 of the 4 barn units were used for analysis. All data were subjected to ANOVA in a crossover with a 2 periods × 2 treatments design, using the MIXED procedure in SAS (edition 9.4, SAS Institute Inc., Cary, NC). Dietary treatment, treatment sequence (i.e., the order in which GS and CS were provided), and period were considered fixed effects. The model included barn unit and week as random factors. Differences were considered significant at $P \leq 0.050$ and tendencies at $0.050 < P \leq 0.100$.

RESULTS

Nutrient Intake, Excretion, and Digestibility

Intake, excretion, and ATTD of nutrients are presented in Table 3. The DMI was not affected by dietary treatment, but due to the major differences in chemical composition between GS and CS (Table 3), intake of most nutrients was affected. The intake of OM tended ($P = 0.067$) to be higher, the intake of crude fat, ADL, and starch were higher ($P < 0.001$), and the intake of N (and subsequently CP) and sugar were lower ($P < 0.001$) for CS compared with GS. The excretion of OM tended ($P = 0.050$) to be higher, and the excretion of NDF, ADF, and starch was higher ($P \leq 0.012$), whereas the excretion of crude fat tended ($P = 0.077$) to be lower, and the excretion of N was lower ($P = 0.014$) for CS compared with GS. The ATTD of crude fat tended ($P = 0.057$) to be higher, whereas that of NDF was lower ($P < 0.001$) for CS compared with GS.

Body Weight and Lactation Characteristics

The BW of the cows receiving CS was lower ($P < 0.001$) than when they received GS (Table 4). Daily milk yield tended ($P = 0.056$) to be lower and FPCM was lower ($P < 0.001$) for CS compared with GS. The latter, combined with an unaffected DMI, resulted in a lower ($P < 0.001$) feed efficiency (kg FPCM/kg DMI) for CS. The content of fat, protein, and urea in milk as well as the yield of milk fat and milk protein (both in g/d) were lower ($P<0.001$), whereas the milk content of lactose was higher ($P = 0.035$) for CS compared with GS.

Chemical Composition and Amount of Manure

At the end of the measurement periods, the manure pits underneath the barn units were filled with (on average) 119 ± 11.1 m^3 manure; on average 121 ± 11.7 m^3 for GS and 116 ± 11.7 m^3 for CS. The chemical composition

Table 3. Intake, excretion, and apparent total-tract digestibility of nutrients in dairy cows receiving a grass silage-based diet (GS) or a corn silage-based diet (CS; all units and numbers measured on barn unit level but given per cow)

Item	GS	CS	SEM	*P*-value
Intake (kg/cow per day unless stated otherwise)				
DM	23.1	22.9	0.29	0.555
OM	20.8	21.3	0.26	0.067
N	0.634	0.495	0.0072	<0.001
Crude fat	0.96	1.01	0.012	<0.001
Gross energy (MJ/d)	429	428	5.3	0.797
NDF	8.47	8.54	0.105	0.484
ADF	5.23	5.18	0.065	0.383
ADL	0.488	0.600	0.0067	<0.001
Starch	2.08	4.78	0.042	<0.001
Sugar	2.33	1.19	0.024	<0.001
Excretion (kg/cow per day)				
DM	6.34	7.57	0.984	0.219
OM	4.53	6.14	0.800	0.050
N	0.523	0.376	0.0564	0.014
Crude fat	0.253	0.186	0.0527	0.077
NDF	1.75	3.56	0.342	<0.001
ADF	1.20	2.36	0.328	<0.001
ADL	0.674	0.807	0.1478	0.133
Starch	0.024	0.044	0.0087	0.012
Apparent total-tract digestibility (% of intake)				
Crude fat	73.5	81.0	5.03	0.057
NDF	79.0	57.5	4.17	<0.001
Starch	98.8	99.1	0.37	0.367

at the start of the measurement periods (i.e., representing the homogenized manure from another barn with which the manure spits were filled) was identical for both diets, except for crude fat content, which tended ($P = 0.074$) to be higher in the manure of CS cows than those of GS cows (Table 5). At the end of the measurement periods (i.e., representing the homogenized manure including the manure from another barn with which the manure pits were originally filled plus the manure produced during the experiment), the chemical composition of the manure produced by the dairy cows was considerably affected by the diet fed. The manure of the CS-fed cows tended ($P = 0.077$) to contain more starch and contained more ($P \leq 0.013$) DM, OM, NDF, and ADF, but contained less ($P \leq 0.034$) total N, as well as organic and inorganic N, and crude fat than the manure produced by GC-fed cows.

Cow-Associated Emissions and Manure Emissions

Table 6 shows the enteric CH_4 emissions and cow-associated CO_2, NH_3, and N_2O emissions, which represent

Table 4. Body weight and lactation characteristics of dairy cows receiving a grass silage-based diet (GS) or a corn silage-based diet (CS)[1]

Item	GS	CS	SEM	*P*-value
BW (kg)	683	672	11.7	<0.001
Lactation characteristic				
Milk yield (kg/d)	26.8	26.4	1.14	0.056
FPCM[2] (kg/d)	30.7	28.4	1.39	<0.001
Milk fat content (g/100 g)	5.05	4.59	0.060	<0.001
Milk protein content (g/100 g)	3.68	3.56	0.025	<0.001
Milk lactose content (g/100 g)	4.42	4.44	0.019	0.035
Milk urea content (mg/dL)	19.5	11.6	1.14	<0.001
Milk fat yield (g/d)	1,355	1,201	65.8	<0.001
Milk protein yield (g/d)	987	927	38.9	<0.001
Milk lactose yield (g/d)	1,197	1,177	46.5	0.103
SCC (×1,000 cells)	244	261	80.1	0.676
Feed efficiency (kg FPCM/kg DMI)	1.33	1.24	0.064	<0.001

[1]All units and numbers measured on individual cow level, except for feed efficiency, which is measured on barn unit level but given per cow

[2]Fat- and protein-corrected milk = (0.337 + 0.116 × milk fat content + 0.06 × milk protein content) × milk yield (kg/d) (CVB, 2018).

Table 5. Chemical composition of manure (g/kg DM, unless stated otherwise) at the start of the measurement periods (i.e., representing homogenized manure from another barn) and at end of the measurement periods [i.e., representing both basis + manure produced by the dairy cows receiving a grass silage-based diet (GS) or a corn silage-based diet (CS)]

Item	Start of measurement periods				End of measurement periods			
	GS	CS	SEM	*P*-value	GS	CS	SEM	*P*-value
DM (g/kg)	87.8	89.7	7.37	0.682	84.4	98.1	4.57	0.013
OM	768	777	11.2	0.333	745	791	4.4	0.004
Nitrogen								
Organic	44.6	42.5	2.29	0.326	48.7	39.3	0.89	0.008
Inorganic	20.2	17.8	1.27	0.266	23.5	16.7	1.23	0.003
Total	64.8	60.2	2.78	0.285	72.2	55.9	2.09	0.004
Crude fat	36.8	39.8	2.77	0.074	38.1	33.4	1.15	0.034
NDF	398	391	20.1	0.604	350	425	7.5	<0.001
ADF	257	259	20.6	0.779	228	281	6.1	<0.001
ADL	77.6	82.4	11.83	0.167	88.3	92.4	1.89	0.135
Starch	7.00	6.49	0.394	0.288	5.63	6.17	0.289	0.077

the emissions measured when the dairy cows were present in the barn units corrected for the emissions from manure (measured when the cows were not present in the barn units). Daily production of CH_4 (g/d) and CH_4 production relative to BW (g/kg BW) were 10.6 and 9.3%, respectively, lower ($P < 0.049$) and CH_4 emission relative to DMI (g/kg DMI) tended ($P = 0.065$) to be 9.4% lower for CS compared with GS. Methane production relative to milk yield (g/kg FPCM) was not affected by dietary treatment. Emissions of CO_2, irrespectively of the unit of expression, were lower ($P \leq 0.015$), whereas the CH_4 to CO_2 ratio was higher ($P = 0.029$) for CS compared with GS. Also, the nitrogenous emissions (i.e., NH_3 and N_2O) were lower ($P < 0.001$) for CS compared with GS, irrespectively of the unit of expression. Production of NH_3 (g/d) was for example 40.4% lower and N_2O production (g/d) was 45.0% lower.

Table 7 shows the measured manure emissions in the barn units when no cows were present. These emissions are expressed as daily production (g/d), but also relative to manure characteristics. For the latter, we discriminate between the total manure present in the manure pit (i.e., homogenized manure present at the start + experimental manure) and solely experimental manure. Manure emissions expressed as a daily pro-

Table 6. Enteric CH_4 emission and cow-associated CO_2, NH_3, and N_2O emissions[1] from dairy cows receiving a grass silage-based diet (GS) or a corn silage-based diet (CS); all units and numbers measured on barn unit level but given per cow

Item	GS	CS	SEM	*P*-value
CH_4				
g/d	413	369	61.7	0.027
g/kg DMI	18.1	16.4	2.79	0.065
g/kg FPCM[2]	13.4	13.4	2.39	0.956
g/kg BW	0.61	0.55	0.094	0.049
CO_2				
g/d	11,865	9,738	1,093.3	<0.001
g/kg DMI	520	430	49.2	<0.001
g/kg FPCM	386	347	37.0	0.015
g/kg BW	17.5	14.6	1.75	<0.001
CH_4-to-CO_2 ratio (× 100)	3.4	3.8	0.36	0.029
NH_3				
g/d	24.8	14.8	1.62	<0.001
g/kg DMI	1.09	0.66	0.075	<0.001
g/kg FPCM	0.81	0.53	0.049	<0.001
g/kg BW	0.036	0.022	0.0027	<0.001
N_2O				
g/d	0.53	0.29	0.195	<0.001
g/kg DMI	0.023	0.013	0.0087	<0.001
g/kg FPCM	0.017	0.010	0.0061	<0.001
g/kg BW	0.0008	0.0004	0.00030	<0.001

[1]Cow-associated CO_2, NH_3, and N_2O emissions are excluding the emissions from manure.

[2]Fat- and protein-corrected milk = (0.337 + 0.116 × milk fat content + 0.06 × milk protein content) × milk yield (kg/d) (CVB, 2018).

duction (g/d) were not statistically tested, because the empty weeks (i.e., weeks without cows present in the barn units) were not well balanced over time and subsequently amount of manure in the manure pits for GS and CS (Figure 1). An effect could be expected based on experiment design alone, where the dairy manure emissions are expected to be higher for CS than for GS. Manure CH_4 emissions were not affected by dietary treatment, except for CH_4 (g/kg excreted OM), which tended to be lower ($P = 0.088$) for CS compared with GS. Manure CO_2 emissions were not affected by dietary treatment, except for CO_2 (g/kg excreted DM), which tended to be lower ($P = 0.098$) for CS compared with GS. Manure NH_3 emissions were not affected by dietary treatment, except for NH_3 (g/kg excreted DM) and NH_3 (g/kg excreted OM), which both tended to be lower ($P \leq 0.092$) for CS compared with GS. Manure N_2O emissions were not affected by dietary treatment.

Enteric CH_4 emission and CH_4 emission from manure represented 95 and 5%, respectively, of the total CH_4 emission measured for both dietary treatments (Figure 3, upper panel). The sum of enteric CH_4 emission and CH_4 emission from manure (Figure 3, lower panel) was lower (383 g/cow per day) for CS compared with GS (431 g/cow per day), due to the difference in enteric CH_4 emission. Enteric and manure CH_4 emissions as well as cow-associated and manure emissions of NH_3 and N_2O, all expressed in CO_2 equivalents (Figure 4), are lower for CS compared with GS.

DISCUSSION

The concentrate composition was identical for both diets, despite the major difference in forage composition. This is not an approach often applied in the dairy sector, where the concentrate composition is formulated to meet the energy and protein requirements for maintenance and milk production of the dairy cows on the complete ration level, and therefore will depend on the forage composition and quality. However, the applied method allowed us to selectively investigate the effect of replacing grass silage by corn silage without having the change in forage composition being confounded with a change in concentrate composition. Not changing the concentrate composition for the protein level of the forages, resulted in a considerably lower dietary CP content and rumen-degradable protein balance (**OEB**; van Duinkerken et al., 2011) for CS (i.e., 135 g of CP/kg of DM and −8 g of OEB/kg of DM, respectively) compared with GS (i.e., 172 g CP/kg DM and 135 g of OEB/kg of DM), whereas dietary NE_L was only slightly different (i.e., 6.6 and 6.7 for CS and GS, respectively).

Although we were able to achieve a maximum effect on N emissions with this dietary strategy (i.e., replacing grass silage with corn silage without changing the concentrate composition), CP intake with CS may have been below the dairy cow requirements. We measured milk urea content (mg/dL), which contains 47% N (Beatson et al., 2019). When using this percentage to convert milk urea content to MUN, we have a MUN value of 9.1 and 5.4 mg/dL for GS and CS, respectively. These values are within the range of MUN values commonly observed in practice (5.0–15.0 mg/dL), but especially the MUN value for CS appears to be lower than the minimum MUN value (10.0 mg/dL) previously indicated to reflect possible shortage of protein (Spek et al., 2013). The estimated intestinal digestible protein (van Duinkerken et al., 2011) still appeared sufficient according to practice. However, the estimated OEB was negative, which is against current recommendations in practice (i.e., OEB of 0). In other words, the cows receiving CS in the current study may have experienced some level of N limitation, although it must be kept in mind that the dietary NE_L as well as DMI were numerically lower for CS compared with GS, which led to a lower NE_L intake. This will be considered when discussing the animal performance results below.

The gas concentrations were measured in ppm and were converted to mg/m^3 using molar volume at the fixed temperature of 20°C and not the actual temperature in the barn units (i.e., not measured). The barn units were located within a large barn, resulting in less temperature fluctuations. Hence, we feel that this applied method resulted in a systemic error only, meaning identical errors for all barn units and for both treatments. The treatment effects are thus well estimated, although the actual emission values might be somewhat deviating compared with when converting ppm to mg/m^3 using the actual temperature in the barn units.

Animal Performance

Although studies differ in experimental design [i.e., some studies replaced grass silage with corn silage without changing the composition of the concentrate (such as in the current study) and other studies changed the ratio between grass silage and corn silage, as well as the composition of the concentrate], many studies reported positive effects of corn silage-based diets on feed intake (e.g., Abrahamse et al., 2008; Kliem et al., 2008; van Gastelen et al., 2015; Günal et al., 2019). The lack of effect on DMI in the current study may be related to the higher starch content of CS compared with GS, which is expected to decrease the ruminal molar proportion of acetate and increase that of propionate and reduce ruminal pH (Bannink et al., 2006). A lower ruminal pH is generally associated with inhibition of fiber fermentation, without affecting starch fermentation, because a

Table 7. Emissions from stored manure only (excluding enteric or cow-associated emissions) of the barn units in which a grass silage-based diet (GS) or a corn silage-based diet (CS) was fed to dairy cows

Item	GS	CS	SEM	*P*-value
CH_4 production (g/d)	9,567	9,753	623.6	ND[1]
CH_4 production related to total manure				
g/m^3 present	94.5	91.7	9.85	0.849
g/kg DM present	1.08	0.93	0.100	0.335
g/kg OM present	1.43	1.18	0.127	0.217
g/kg C present	2.56	2.19	0.237	0.313
g/kg total N present	15.9	16.3	1.71	0.863
g/kg organic N present	23.3	23.2	2.42	0.974
g/kg inorganic N present	50.1	55.2	6.04	0.570
CH_4 production related to experimental manure				
g/excreted m^3	484	320	100.7	0.291
g/kg excreted DM	5.66	2.63	1.136	0.109
g/kg excreted OM	7.67	3.76	1.359	0.088
g/kg excreted C	12.7	7.1	2.30	0.134
g/kg excreted total N	76.7	57.0	16.64	0.435
g/kg excreted organic N	114.4	82.1	24.36	0.385
g/kg excreted inorganic N	233	189	52.4	0.568
CO_2 production (g/d)	167,248	180,903	21,158.0	ND[1]
CO_2 production relative to total manure				
g/m^3 present	1,663	1,660	203.1	0.990
g/kg DM present	19.0	16.9	2.24	0.468
g/kg OM present	25.1	21.4	2.84	0.340
g/kg C present	45.1	39.9	5.44	0.430
g/kg total N present	280	298	39.1	0.675
g/kg organic N present	411	423	56.4	0.838
g/kg inorganic N present	887	1,010	142.0	0.341
CO_2 production relative to experimental manure				
g/excreted m^3	8,732	5,640	1,894.4	0.292
g/kg excreted DM	102	43	21.3	0.098
g/kg excreted OM	138	65	26.7	0.103
g/kg excreted C	228	124	44.9	0.150
g/kg excreted total N	1,388	1,009	309.2	0.420
g/kg excreted organic N	2,067	1,442	446.8	0.361
g/kg excreted inorganic N	4,235	3,398	1,012.4	0.563
NH_3 production (g/d)	197	184	20.8	ND[1]
NH_3 production related to total manure				
g/m^3 present	1.94	1.74	0.256	0.564
g/kg DM present	0.022	0.018	0.0024	0.181
g/kg OM present	0.029	0.022	0.0031	0.120
g/kg C present	0.052	0.413	0.0057	0.139
g/kg total N present	0.324	0.307	0.0401	0.746
g/kg organic N present	0.476	0.436	0.0563	0.577
g/kg inorganic N present	1.02	1.04	0.144	0.912
NH_3 production related to experimental manure				
g/ excreted m^3	9.70	6.15	2.045	0.265
g/kg excreted DM	0.113	0.051	0.0219	0.092
g/kg excreted OM	0.154	0.073	0.0257	0.068
g/kg excreted C	0.254	0.137	0.0439	0.109
g/kg excreted total N	1.51	1.10	0.343	0.424
g/kg excreted organic N	2.26	1.59	0.499	0.379
g/kg excreted inorganic N	4.59	3.64	1.100	0.543
N_2O production (g/d)	10	11	1.5	ND[1]
N_2O production related to total manure				
g/m^3 present	0.0984	0.1059	0.01871	0.774
g/kg DM present	0.0011	0.0011	0.00017	0.898
g/kg OM present	0.0015	0.0014	0.00021	0.756
g/kg C present	0.0026	0.0025	0.00036	0.881
g/kg total N present	0.0164	0.0188	0.00272	0.552
g/kg organic N present	0.0239	0.0267	0.00371	0.608
g/kg inorganic N present	0.0523	0.0636	0.01029	0.464
N_2O production related to experimental manure				
g/excreted m^3	0.536	0.372	0.1640	0.497
g/kg excreted DM	0.0061	0.0031	0.00167	0.252
g/kg excreted OM	0.0082	0.0044	0.00205	0.234
g/kg excreted C	0.0135	0.0082	0.00337	0.310

Continued

Table 7 (Continued). Emissions from stored manure only (excluding enteric or cow-associated emissions) of the barn units in which a grass silage-based diet (GS) or a corn silage-based diet (CS) was fed to dairy cows

Item	GS	CS	SEM	*P*-value
g/kg excreted total N	0.0837	0.0663	0.02705	0.625
g/kg excreted organic N	0.125	0.096	0.0397	0.582
g/kg excreted inorganic N	0.255	0.218	0.0851	0.733

[1]Not determined. The empty weeks (i.e., without cows present in the barn units) were not well balanced over time and subsequently amount of manure in the manure pits for GS or CS, and an effect could be expected based on experiment design alone.

low pH has a negative effect on rumen microbiome, in particular on fiber fermenting bacteria. It is recognized that the growth of cellulolytic bacteria and fiber digestion starts to become compromised at pH 6.0 to 5.8 (Mould and Ørskov, 1983; Hoover, 1986). This may have occurred in the present study as well, where the ATTD of NDF was reduced by 21.5% for CS compared with GS, although a lower NDF digestibility for corn silage compared with grass silage is also to be expected based solely on their NDF degradation characteristics with corn silage NDF intrinsically being less degradable than grass silage NDF. The ATTD of starch was similar between the 2 diets. This may have favored propionate production (van Soest, 1994), which can promote satiety (Allen, 2000; Stocks and Allen, 2012) and may explain the absence of an increased DMI with CS in the present study. We should, however, be aware that dietary CP content also differed between GS and CS, which may explain the lack of effect on DMI. However, in agreement with the results of the current study, Leonardi et al. (2003) as well as Colmenero and Broderick (2006) also did not observe an effect of dietary CP content on DMI of dairy cows when dietary CP was increased from 16.1 to 18.8% and from 13.5 to 19.4%, respectively. Reynolds et al. (2010) on the other hand demonstrated that DMI increased with increasing dietary CP content (from 14.0 to 18.0%), although the effect differed with forage type (response present for a corn silage-based diet, but absent for a grass silage-based diet).

Although Reynolds et al. (2010) reported no effect of dietary CP content (14.0, 16.0, and 18.0%) on daily milk yield, as did Leonardi et al. (2003; CP content of 16.1 and 18.8%), replacing grass silage with corn silage in the current study tended to decrease milk production, partially because of the low dietary CP content (and intake) and partially because of the numerically lower NE_L intake for CS. Colmenero and Broderick (2006) observed a tendency for a quadratic response in milk yield, where milk yield increased when dietary CP content increased from 13.5 to 16.5% but declined when dietary CP content continued to increase to 19.4%. This agrees with the multivariate regression analysis conducted by NRC (2001) and Ipharraguerre and Clark (2005). The latter reported a curvilinear relation between daily milk yield and dietary CP content with the milk production response being larger when the dietary CP content increased at the low range (i.e., from 15 to 16%) than that with the dietary CP content increased at the high range (i.e., from 19 to 20%). However, Ipharraguerre and Clark (2005) did report that the considerable variation in the relationship between the dietary CP content and daily milk yield was accounted for by the source of CP. Additionally, Law et al. (2009) observed beneficial effects on milk production with increasing dietary CP content from 14.4 to 17.3%, but for cows in early lactation only (not for cows in late lactation). The cows in the current experiment were in mid lactation (average 160 DIM), thus the effect of

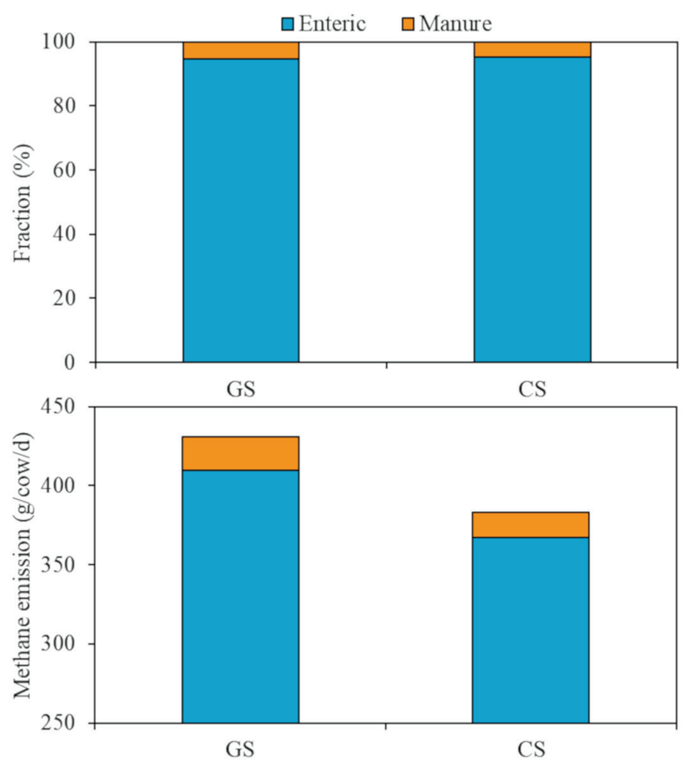

Figure 3. Enteric CH_4 emission (blue) and manure CH_4 emission (orange) as fractions relative to total CH_4 emission (upper panel) and in g/d (lower panel) of the grass silage-based diet (GS) and the corn silage-based diet (CS).

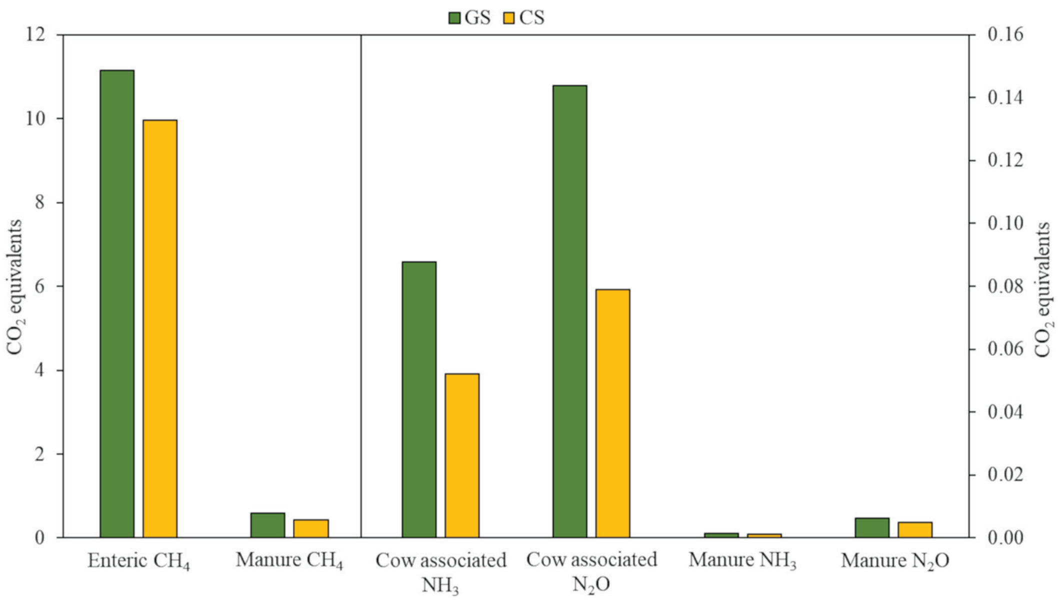

Figure 4. Greenhouse gas balance in CO_2 equivalents of enteric and manure CH_4 emissions (on left y-axis) and of cow-associated and manure NH_3 and N_2O emissions (on right y-axis) of the grass silage-based diet (GS; green bars) and the corn silage-based diet (CS; yellow bars). Enteric CH_4 as well as cow-associated NH_3 and N_2O refer to emissions measured from cows directly (excluding emissions from manure in the manure pits beneath the slatted floor). Manure CH_4, NH_3, and N_2O emissions refer to manure emissions only (in absence of cows).

replacing grass silage with corn silage on milk production may have been somewhat different from that of cows in early lactation.

The decreased FPCM for CS is the result of a tendency for reduced milk yield in combination with a considerable drop in milk protein content as well as milk fat content. Generally, an increased intake of readily digestible carbohydrates, such as starch, and a decreased intake of fibrous components is associated with a reduction in milk fat production (Lock and Shingfield, 2004; Nielsen et al., 2006). This is likely the result of the decreased molar proportion of acetate and increase in that of propionate (Bannink et al., 2006), which relates to a depression of milk fat content through different mechanisms (Bauman and Griinari, 2003). For example, an increased rumen production of propionate and enhanced hepatic rates of gluconeogenesis cause an increase in circulating insulin, which can result in an insulin-induced shortage of precursors for mammary synthesis of milk fat. Additionally, short- and even-chain fatty acids (i.e., <16C) are synthesized de novo in the mammary gland primarily from acetate (Bauman and Griinari, 2003) and indirectly from butyrate (Dijkstra et al., 2011), originating from ruminal fermentation. Therefore, an increased intake of starch, potentially coupled with a decreased intake of rumen-degradable NDF, is generally associated with a reduction in milk fat (Lock and Shingfield, 2004; Nielsen et al., 2006). The decreased dietary CP content for CS compared with GS might also have played a role in the decreased milk fat content and milk fat yield observed, although results in literature are inconsistent [e.g., no effect found by Mutsvangwa et al. (2016), but relations between dietary CP content and milk fat content reported by Leonardi et al. (2003) and Colmenero and Broderick (2006)].

The decreased milk protein content with CS is contrary to the findings reported by other studies where replacement of grass silage by corn silage resulted in an increased milk protein content (e.g., Abrahamse et al., 2008; Kliem et al., 2008; van Gastelen et al., 2015). The decreased milk protein content (−3.3%; −0.12 g/100 g) and milk protein yield (−6.1%; −60 g/d) observed in the current study for CS may be related to the negative dietary OEB value and the lower dietary CP content (−37 g/kg DM) in CS and associated lower CP intake (−870 g/d), which may have resulted in less feed N available for milk protein synthesis. In support of this is the low milk urea content for CS compared with average values encountered in practice (comparing more to 20 mg/dL as established for GS; Šebek and van Bruggen, 2021). Furthermore, other studies have reported reduced ATTD of N (e.g., Günal et al., 2019) when grass silage was replaced by corn silage. The ATTD of N could not be measured in the present study, because total collection of urine or feces separately was not possible, but it is to be expected that ATTD of N was lower for CS due to the lower dietary CP intake and available metabolizable protein (Bannink et al., 2018).

The synthesis of lactose requires glucose (Cant et al., 2002), and there is considerable capacity for starch digestion in the small intestines via enzymatic hydrolysis resulting in increased glucose supply to the animal (Reynolds, 2006). This is supported in the present study, where we see a small increase in milk lactose content when cows received CS.

Enteric Methane and Cow-Associated CO_2 and Nitrogenous Emissions

The measured enteric CH_4 production and CH_4 production relative to feed intake for both dietary treatments were within the range of CH_4 measurements reported in literature (Appuhamy et al., 2016). As expected, replacing grass silage with corn silage resulted in decreased enteric CH_4 emissions. This agrees with many studies, including Staerfl et al. (2012), Brask et al. (2013), and van Gastelen et al. (2015), and can be explained by 4 factors. First, fermentation of starch favors ruminal production of propionate at the expense of acetate, which reduces H_2 availability for CH_4 production and activity of rumen methanogens (Van Kessel and Russell, 1996; Hook et al., 2011). Second, we observed a lower ATTD of NDF with CS. This means that ruminal fiber fermentation was less with furthermore the starch partly escaping rumen fermentation, leading to less H_2 formation and subsequently methanogenesis (Jayanegara et al., 2012). Third, the small difference in dietary fat content between GS and CS (+3 g/kg DM) may have helped decreasing enteric CH_4 emissions, because fat is not fermented in the rumen and may inhibit metabolic activity of fibrolytic bacteria and methanogens (Martin et al., 2010), resulting in less H_2 formation as substrate for methanogenesis (Beauchemin et al., 2008), and fat can function as a H_2 sink through ruminal biohydrogenation (Jenkins et al., 2008) although this effect is normally minor. Fourth, the difference in dietary CP content (38 g/kg DM difference, with dietary CP content of CS below protein requirements of the dairy cows) may have affected CH_4 emissions, although the reported effect of dietary CP content on enteric CH_4 emission is variable in literature. Ellis et al. (2009) found a positive relationship between dietary CP content and CH_4 emission in beef cattle, whereas Reynolds et al. (2010) did not observe differences in CH_4 yield with different dietary CP contents. Methane emission relative to FPCM was not affected in the present study and is likely the result of a decreased CH_4 production (in g/d) in combination with a decreased FPCM with CS compared with GS, where the decreased FPCM is due to the large difference in dietary CP content between the 2 diets (as discussed above).

The reported cow-associated CO_2 production is in line with the calculated CO_2 production (in g/d) when applying the heat production equation described by CIGR (2002), based on daily milk production (kg/d), BW (kg), and duration of pregnancy (d), and using a fixed CO_2 production of 185 L/h per heat production unit (i.e., 1,000 W of the total animal heat production at 20°C). The decrease in cow-associated CO_2 production for CS is likely the direct result of a lower BW and milk production compared with GS. In addition, CO_2 formation in the rumen may have played a small role, because relatively more propionate was likely produced with CS (without CO_2 loss) at the expense of acetate and butyrate production (with CO_2 production). Contrary though, assuming that in methanogenesis 1 mol CO_2 is used to form 1 mol CH_4 (McAllister and Newbold, 2008), the 1.7 g of CH_4/kg of DMI decrease with CS compared with GS, corresponds in theory with an increase of 4.7 g of CO_2/kg of DMI with CS. This increase however appears neglectable in comparison with the sum of different processes affecting CO_2 emission.

The reported cow-associated NH_3 emissions in the present study represent the NH_3 emission directly originating from urea excreted in urine at the moment of urination (i.e., the moment that urine comes into contact with feces and feces-fouled surfaces). Several studies have shown that an increased dietary intake of N leads to a moderate, linear increase of fecal N excretion and milk N secretion combined but a much more pronounced linear (Kebreab et al., 2010) or exponential (Castillo et al., 2000; Kebreab et al., 2001) increase in urinary N excretion. The majority of urinary N is excreted in the form of urea, which is rapidly hydrolyzed by microbial urease activity to NH_3 (Misselbrook et al., 2005). Emissions of NH_3 therefore begins directly following urine deposition in the dairy barn unit, with urine coming in contact with feces and feces-fouled surfaces such as the barn unit floor. Reducing urinary N excretion, mediated through a reduced dietary CP content, should thus lead to reductions in NH_3 emissions. Several studies using laboratory chamber systems measuring NH_3 emissions from manure have shown that reduced NH_3 emissions are directly associated with lower dietary CP content (e.g., Paul et al., 1998; Külling et al., 2001). Other studies showed the same relation at the barn level (e.g., Edouard et al., 2019; van Duinkerken et al., 2005). In the present study, the dietary CP content was 22% lower for CS (135 g/kg DM vs. 172 g/kg DM for GS). Because of the unaffected DMI, this difference in dietary composition resulted in decreased N excretion (−28%, particularly by a reduced urine N excretion) and subsequently a lower N content in manure (−22%). Hence, expectedly, the cow-associated NH_3 emissions measured in the present study decreased by 38.6%.

Cow-associated N_2O emissions are considered small relative to other sources of N_2O emissions (IPCC, 2006), such as nitrification and denitrification (i.e., 2 soil microbial processes) following manure application (de Klein and Eckard, 2008), livestock bedding, solid manure stores, and the surface layer of stored slurry (Sommer et al., 2000; Chadwick, 2005). Veldman and Oenema (1997) previously demonstrated that N_2O emissions from fresh cow urine applied to a stable floor were negligible, and Kaspar and Tiedje (1981) showed that only small trace amounts of N_2O were produced during dissimilatory reduction of NO_2^- to NH_4^+ in the rumen. The cow-associated N_2O yields measured for GS and CS in the present study are well within the range reported in literature. Petersen et al. (2015), for example, reported cow-associated N_2O emissions of 1.5 mg N_2O/kg DMI for dairy cows receiving a 15.2% CP (control) diet. Mitloehner et al. (2009) reported cow-associated N_2O emissions of 480 mg N_2O/animal per day for dairy cows fed a 17.2% CP diet with a DMI of 11.4 kg/d, which means 42 mg of N_2O/kg of DMI. The main driver for N_2O emissions includes N availability (Parker et al., 2018; Petersen et al., 2015; Selbie et al., 2015). The authors of the Dairy Gas Emissions Model proposed a cow-associated N_2O emission rate for dairy cattle of 0.08% of daily N intake (Rotz et al., 2016), which, for the present study, would result in 0.51 and 0.40 g N_2O/cow per day for GS and CS, respectively. This was indeed measured for GS, but the measured N_2O production for CS was 27% lower. The lower dietary CP content and subsequently decreased (particularly urine) N excretion (−28%) as well as N content in manure (−22%) for CS, seems to have caused cow-associated N_2O emissions to decrease on average by 43.7% in comparison to GS.

Manure Composition

At the start of both periods, the manure pits below the barn units were filled up to 50 cm in height with homogenized manure from another barn of the research facility. Despite some small numerical differences, the composition of the manure at the start of the periods was not statistically different between GS and CS. Therefore, our aim to ensure similar manure composition at the start of the measurement periods was achieved.

The quantity as well as the composition of OM excreted by the dairy cows is determined by the diet (Massé et al., 2016; Hassanat and Benchaar, 2019). In the present study, the quantity of manure OM was higher for CS than for GS, which is likely caused by the increased OM intake (tendency for +0.5 kg/d) and perhaps, although not measured in the present study, in combination with a decreased ATTD of OM. A decreased ATTD of OM is likely because the increase in OM excretion (+1.23 kg/d) was considerably larger than the increase in OM intake and seems to be largely caused by the increase in excreted NDF. An increased excretion of microbial OM due to hindgut fermentation of starch may also have contributed to this. Corresponding to the present results, Brask et al. (2013) also reported a decreased ATTD of OM when grass silage was replaced by corn silage. In the present study, the composition of manure OM was affected by dietary treatment. First, the NDF content in manure was higher for CS compared with GS, as result of a considerably lower ATTD of NDF (NDF intake unaffected), and consequently increased NDF excretion. At the end of the experimental periods, NDF represented approximately 47% and 54% of the manure OM for GS and CS, respectively. Second, the starch content in manure DM was higher for CS compared with GS, but the amount remained very small (0.8% of the manure OM for both diets). Third, the total N content in manure was 22% lower for CS compared with GS, where N represented approximately 10% and 7% of the manure OM for GS and CS, respectively. This was caused by the considerably lower dietary CP content and consequently lower N relative to OM excretion for CS.

Methane and Nitrogenous Emissions from Manure

The extent in which CH_4 emission of manure storage contributes to the total CH_4 emissions depends on many factors, including manure management practices, such as storage duration, emptying frequency, and removal of residual sludge, but also on the storage conditions and manure temperature and the chemical composition of the manure (Chadwick et al., 2011). In the present study, CH_4 emissions from manure represented only 5% of the total CH_4 emissions measured (Figure 3), a considerably smaller percentage according to the IPCC (2006), which can be explained by 2 factors. First, both periods lasted 7 wk in total, where the manure emission were measured 4 to 7 wk after the start of the periods (Figure 1). In terms of length of manure storage, the present study can be considered a short-term study. Hindrichsen et al. (2005) measured enteric and slurry methanogenesis after 7 and 14 wk of slurry storage upon feeding different types of dietary carbohydrates. They reported that CH_4 emissions from manure ranged between 5.2 and 10.8% of total CH_4 emissions at 7 wk of storage, and between 16.0 and 21.9% of total CH_4 emissions at 14 wk of storage. This is in line with the percentage found in the present study. Second, the manure pits below the barn units were filled up to 48 ± 2.8 cm in height (i.e., 67 ± 3.9 m^3; realized) with homogenized external manure introduced at the start of both periods. This manure represented a rather large proportion of the total manure present in the pits at the moments that manure emissions were measured. For example, the manure pits were (on average; taken both treatments, both measurement period, and all barn units into account) filled up to 86 ± 8.1 cm in height (i.e., 119 ± 11.1 m^3) at the end of the periods, which means that the experimental manure (produced during the experiment; representing the dietary treatments) represented only 44% of the total manure present at the end of the trial. This percentage was even smaller at the moment of that manure emissions were measured, which may have obscured the observation of significant treatment differences in experimental manure emissions (which did differ numerically), however it is no explanation for the small contribution of experimental manure to total CH_4 emission.

The available amount of nutrients in manure for microbial fermentation directly influences the magnitude of CH_4 emissions during manure storage. As shown above, both quantity and composition of manure OM were affected by the dietary treatments in the present study. However, despite the higher OM, NDF, and starch content in manure (latter tendency only) for CS, CH_4 emissions from total manure were not affected. Only numerical differences could be demonstrated for CH_4 emissions from experimental manure (except a tendency for lower CH_4 per kilogram of excreted OM). Methane can only be generated during anaerobic decomposition of OM (Gilroyed et al., 2011). During the present experiment, the content of the manure pits was not mixed and hence, the manure produced during the experiment (representing the dietary treatment) generally formed the surface layer of the manure in the manure pits. This may not have provided sufficient anaerobic conditions or a sufficiently active methanogen population yet to let become CH_4 production distinctive between treatments. The homogenized manure on the other hand, formed the bottom-layer of the manure in the manure pits, with a relatively much more anaerobic zone allowing the development of CH_4 production. Therefore, the measured manure CH_4 emissions in the present study may predominantly have originated from the homogenized manure rather than experimental manure.

As described above, to estimate manure emissions we used the data from d 3 onward after cows were removed from the barn units, because we observed that the emissions of NH_3 and N_2O reached a stable plateau after d 2. This is consistent with the time required for complete hydrolysis of the urea content in excreted urine, which precedes NH_3 volatilization. Whitehead and Raistrick (1993) demonstrated that complete hydrolysis at 15°C would occur within 10 to 15 d, but Muck (1982) reported much faster hydrolysis of urea on dairy barn floors, with >95% urea decomposed within 6 h at 30°C and within 24 h at 10°C. The N_2O emissions measured in the present study are relatively low. This in agreement with Sommer et al. (2000) who stated that N_2O emissions from slurry or liquid manure with no surface cover is negligible. Only stored slurry with a natural crust is considered a substantial source of N_2O emissions (Sommer et al., 2000; VanderZaag et al., 2009), because the presence of a surface crust can provide partial aerobic/anaerobic conditions in the crust, resulting in nitrification with N_2O generation as a result (Sommer et al., 2000). Although cattle slurry may form this crust naturally, it hardly did in the present study because of the short-term character of the study as is demonstrated by the very low N_2O emissions from manure (<0.001% of excreted N).

Nitrogenous emissions from manure depend on N availability in manure, as discussed above. The N content in manure was considerably lower for CS compared with GS, yet the nitrogenous emissions from manure were not affected by dietary treatment. Interestingly though, the nitrogenous emissions relative to the total manure present in the manure pit were both numerically and statistically similar. However, when expressed relative to the experimental manure (i.e., produced during the study, representing the dietary treatments), nitrogenous emissions from manure were numerically much lower for CS compared with GS. This shows that a difference in dietary CP content can, in principle, result in much lower NH_3 and N_2O emissions from manure, with NH_3 emissions being the predominant N emissions by far. These results are understandable as the N emissions mainly (but not only as also degradation of manure OM may deliver NH_3; Dijkstra et al., 2018) originate from excreted urine N that is highly affected by dietary CP content.

Greenhouse Gas Balance

To directly compare the difference emissions (CH_4 vs. nitrogenous emissions) as well as the different sources of emissions (enteric and cow associated vs. manure), we have converted all emission into CO_2 equivalents (Figure 4). Both enteric CH_4 emission and manure CH_4 emission (numerically) were smaller for CS compared with GS, indicating that there was no trade-off between enteric and manure CH_4 emission (in contrast a small synergy was observed numerically). Additionally, enteric CH_4 emissions as well as cow-associated NH_3 and N_2O emissions were lower for CS compared with GS, with the manure emissions showing a similar pattern, indicating that there was a synergy effect of exchange of grass silage corn silage for CH_4 and nitrogenous emissions.

CONCLUSIONS

Exchanging grass silage with corn silage, without changing the composition of the concentrate, resulted in large dietary composition difference, particular in dietary N and starch. Via different processes, all related to the intake of nutrients, enteric CH_4, as well as cow-associated NH_3 and N_2O emissions were 11, 40, and 45% lower, respectively, for CS compared with GS. Enteric CH_4 emission and CH_4 emission from manure represented 95 and 5%, respectively, of total CH_4 emissions with the small contribution by manure probably due to the short-term character of the study. Overall, there was no trade-off between enteric CH_4 emission and manure CH_4 emission. A strong synergy was observed for CH_4 and nitrogenous emissions when grass silage was exchanged for corn silage, as a direct consequence of the large difference in dietary CP content. This synergy might have been absent or very small if GS and CS were kept isonitrogenous. The compromised cow performance that was observed for the corn silage-based diet can probably be prevented largely by optimizing diet formulation, only partly compensating these potent mitigating effects.

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