Feeding up to 91% concentrate to Holstein and Jersey dairy cows: Effects on enteric methane emission, rumen fermentation and bacterial community, digestibility, production, and feeding behavior

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ABSTRACT

Due to climate change, periods of drought might be longer and occur more frequently, which challenges roughage production and requires changed feeding of dairy cattle by increasing the grain content of the diet. This study investigated the effect of diets with concentrate proportions up to 91% of dry matter on dry matter intake (DMI), milk production, enteric methane emission, rumen fermentation, rumen bacterial community structure, nutrient digestibility, and feeding behavior of Holstein and Jersey dairy cows. Twelve Danish Holstein and 12 Danish Jersey cows were fed ad libitum with one of 3 total mixed rations differing in concentrate proportion in a continuous design with staggered approach over 19 to 29 d. Dietary concentrate proportions were 49% (C49), 70% (C70), and 91% (C91) on dry matter basis, and were based on increasing proportions of chopped barley straw, dried beet pulp, barley, NaOH-treated wheat, dried distillers grain, and rapeseed cake at the expense of grass/clover silage, corn silage and soybean meal. Cows were adapted to the diets over a 12- to 19-d period, before rumination activity was measured over 3 d. Subsequently, spot samples of feces were collected for digestibility determination over 2 d, and gas exchange was measured on the last 3 d of the experimental period. Shortly after chamber stay, rumen liquid was collected using an oro-ruminal device. Dry matter intake was higher for Holstein than Jersey. Methane emissions (all expressions) were affected by the interaction between breed and diet. Methane per kilogram of DMI was lowered by 18 and 48% for Holstein fed C70 and C91, respectively, compared with C49, whereas this was 17 and 22% respectively for Jersey. Rumen propionate molar proportion increased more, rumen bacterial community was less diverse, and rumination time and rumination chews relative to DMI reduced less for Holstein than for Jersey cows with increasing concentrate level. In conclusion, Holstein dairy cows responded stronger to increased dietary concentrate level regarding methane mitigation, changes in rumen VFA profile, and effect on the rumen bacterial community structure than Jersey cows, whereas Jersey cows responded stronger with regard to rumination time and rumination chews (per kilogram of DMI and per kilogram of neutral detergent fiber intake) than Holstein cows. Thus, diets high in concentrates are a less effective methane mitigation strategy for Jersey than for Holstein.

Key words: climate change, dairy cattle, greenhouse gases, methane mitigation

INTRODUCTION

The agricultural sector is faced by challenges related to global warming and climate change, which affect human and animal food security. Changing climatic conditions, such as unexpected seasonal droughts during the growing season, become more frequent and negatively affect the quality and quantity of roughage production (Rojas-Downing et al., 2017). Challenged roughage production requires a changed feeding of dairy cattle in the short term and potentially also in a long-term perspective. For example, temporary increases in the concentrate content of rations could substitute grass silage and corn silage when availability is low. Obviously, also the production of crops for concentrate can be negatively affected by drought, but usually crop yield will not be affected simultaneously in the world. Because transportation of concentrate from other parts of the globe is easier than transportation of roughage, importing concentrate is a potential way to overcome shortage of feed. Depending on the level and composition of concentrate, especially with regard to the level of readily fermentable carbohydrates, in the ration, DMI, and milk yield increase with increasing concentrate proportion (Huhtanen and Hetta, 2012; Olijhoek et al., 2018), whereas, digestibility of NDF
might be reduced (Nousiainen et al., 2009; Olijhoek et al., 2018). In addition, enteric methane emission declines, especially when concentrate inclusion is above 35 to 40% (Sauvant and Giger-Reverdin, 2009). Thus, rations high in concentrate proportion will reduce enteric methane mitigation.

The effects of feeding diets moderately high in concentrate proportion, especially grains (starch), on methane production of dairy cattle is well established, whereas the effect of feeding only concentrate combined with a small amount of fiber from straw is not well studied. Studies investigating high concentrate diets in relation to methane emission for dairy cows have included concentrate up to 72% of DM (Ferris et al., 1999; Agle et al., 2010; Aguerre et al., 2011; Olijhoek et al., 2018) and have mainly focused on the Holstein breed. Jersey is another widely used dairy cattle breed in some countries and differs in gastrointestinal tract size and physiology from larger cattle breeds (Aikman et al., 2008; Beecher et al., 2014). This difference suggests that the same diet might differ in effectiveness between breeds. Previously, we demonstrated that enteric methane emission was reduced to a larger extent for Holstein than Jersey cows when increasing forage-to-concentrate ratio from 68:32 to 39:61 (Olijhoek et al., 2018). Here, we study the effect of 3 diets containing up to 91% concentrate on DM basis. This intensive study is a follow-up to a production study by Borsting et al. (2019), who investigated feed intake and production performance of Holstein and Jersey cows fed 5 rations differing in concentrate proportion and type of concentrate. Three of the 5 rations were subsequently included in the current study to investigate DMI, milk production, enteric methane emission, rumen fermentation, rumen bacterial community structure, nutrient digestibility, and feeding behavior in the same 2 breeds. The 3 diets were based on increasing levels of starch, mainly from rolled barley and NaOH-treated whole kernel wheat, and decreasing levels of NDF. Borsting et al. (2019) showed the largest decrease in milk fat percentage and in rumen acetate:propionate (A:P) ratio for this type of concentrate, which indicates a large effect on rumen fermentation. The aim of this study was to examine the effect of diets with varying concentrate proportions up to 91% of DM on DMI, milk production, enteric methane emission, rumen fermentation, rumen bacterial community structure, nutrient digestibility, and feeding behavior of Holstein and Jersey dairy cows. We hypothesized that feeding increased dietary proportions of concentrate to dairy cows will decrease enteric methane emission, A:P ratio in rumen liquid, total-tract digestibility of nutrients, rumination time, and rumen bacterial diversity, with more pronounced effects for Holstein than Jersey.

**MATERIALS AND METHODS**

**Experimental Design**

The experiment was conducted at the Danish Cattle Research Centre (AU Foulum, Tjele, Denmark) and in accordance with the guidelines of the European Union directive 2010/63/EU and current Danish legislation on animal experimentation (law no. 474, May 14, 2014; license no. 2018-15-0201-1495). Twenty-four dairy cows (12 Danish Holstein and 12 Danish Jersey) were fed one of 3 diets (4 Holstein and 4 Jersey per diet) differing in concentrate proportion in a continuous design with staggered approach. To allocate the diets, cows were divided into 2 blocks of 3 cows within breed and parity (12 primiparous and 12 multiparous) according to DIM (8 blocks in total). Within block, cows were allocated randomly to a specific diet that was fed throughout the experiment. Next, animals were rearranged into 6 new blocks of 4 animals to have 1 cow from each block to be allocated to 1 of 4 respiration chambers available. Each new block consisted of 2 Holstein cows and 2 Jersey cows, and 1 primiparous and 1 multiparous cow (second or third parity) for each breed. The first 4 blocks of cows started the experimental feeding on the same day and the next 2 blocks started the experiment 13 d later. There was capacity to measure feeding behavior in 8 cows at a time. Two of the first 4 blocks began these measurements after 12 d of adaptation, and the remaining 2 of these blocks after 19 d of adaptation, whereas the 2 blocks, that started the experimental feeding later, were measured after 12 d of adaptation. Feeding behavior of all cows were measured for 3 d. Spot samples of feces were collected for digestibility determination over 2 d beginning 14 d after start of the experimental feeding for all cows. Gas exchange was measured using 4 respiration chambers on the last 3 d of the experimental period for each block of 4 cows, resulting in onset of these measurements 16, 19, 23, or 26 d after start of experimental feeding. At the end of the chamber measurements, rumen liquid was collected once. One primiparous Jersey cow on a diet containing 49% concentrate had to be removed from the experiment due to difficulties with milking and was replaced by another cow, which went directly to the 49% concentrate diet 11 d into the experiment, equivalent to 5 d before feces sampling and 10 d before it was moved to the respiration chamber. Feeding behavior and digestibility were not measured for this cow.

**Animals, Diets, and Feeding**

At the start of the experiment, Holsteins were on average (±SD) 140 ± 28 DIM with a milk yield of 37.8
titanium dioxide was added (TiO₂; 1.25 g/kg of dietary premix by DLG (Aarhus, Denmark) to which also and a standard concentrate mixture, were mixed into dried beet pulp, barley, NaOH-treated wheat, dried distillers grain, and rapeseed cake were included at the expense of grass/clover silage, corn silage, and soybean distillers grain. With increasing concentrate proportion in the diet C49 and C70, and only chopped barley straw in diet C91. With increasing concentrate proportion in the diet, increasing proportions of chopped barley straw, dried beet pulp, barley, NaOH-treated wheat, and chopped barley straw for diet C49 and C70, and only chopped barley straw in diet C91. With increasing concentrate proportion in the diet, increasing proportions of chopped barley straw, dried beet pulp, barley, NaOH-treated wheat, and standard concentrate mixture, were mixed into a premix by DLG (Aarhus, Denmark) to which also titanium dioxide was added (TiO₂; 1.25 g/kg of dietary DM). Titanium dioxide served as an external marker to determine nutrient digestibility. The DM concentration of the diets was adjusted by the addition of water to obtain similar DM contents between diets (approximately 400 g/kg of fresh matter). The increase from 49 to 91% concentrate in DM led to an increase in starch from 173 to 223 g/kg of DM, and a decrease in NDF from 306 to 248 g/kg of DM. At the same time, there was a slight increase in fat content from 36 to 42 g/kg of DM, and a slight increase in CP content from 159 to 171 g/kg of DM.

Cows receiving diet C49, were fed this diet from d 1 in the experiment. Adaptation to diet C70 and C91 was achieved gradually by mixing with decreasing proportions of diet C49 and increasing proportions of C91. For diet C70, C49 constituted 83% of DM at d 1 and 2, 67% of DM at d 3 and 4, and 50% of DM at d 5 and onward. For adaptation to diet C91, C49 constituted 67% of DM at d 1 and 2, 33% of DM at d 3 and 4, and 0% from d 5 and onward.

**Sampling and Measurements**

Feed intake was monitored daily throughout the experiment. Dry matter content of TMR and feed residues was determined for all days allocated to determination of digestibility, feeding behavior, and gas exchange. Samples of TMR were collected on d 4 during the experiment: on d 13 and 14 after experimental onset for the first 4 blocks of cows (16 animals) and on d 14 and

**Table 1. Dietary and chemical composition of the diets (g/kg of DM, unless stated otherwise)**

<table>
<thead>
<tr>
<th>Item</th>
<th>C49</th>
<th>C70</th>
<th>C91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary concentrate proportion (%)</td>
<td>49</td>
<td>70</td>
<td>91</td>
</tr>
<tr>
<td>Dietary composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary growth grass/clover silage</td>
<td>113</td>
<td>56.5</td>
<td>0.0</td>
</tr>
<tr>
<td>First regrowth grass/clover silage</td>
<td>142</td>
<td>71.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Corn silage</td>
<td>243</td>
<td>121</td>
<td>0.0</td>
</tr>
<tr>
<td>Barley straw</td>
<td>12.6</td>
<td>50.2</td>
<td>87.9</td>
</tr>
<tr>
<td>Concentrate mixture^2</td>
<td>109</td>
<td>109</td>
<td>109</td>
</tr>
<tr>
<td>Dried beet pulp</td>
<td>120</td>
<td>160</td>
<td>130</td>
</tr>
<tr>
<td>Barley</td>
<td>112</td>
<td>121</td>
<td>110</td>
</tr>
<tr>
<td>Wheat, NaOH treated</td>
<td>0.0</td>
<td>77.4</td>
<td>155</td>
</tr>
<tr>
<td>Dried distillers grain</td>
<td>0.0</td>
<td>68.7</td>
<td>138</td>
</tr>
<tr>
<td>Rapeseed cake</td>
<td>78.6</td>
<td>106</td>
<td>134</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>53.8</td>
<td>27.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Molasses (sugarcane)</td>
<td>4.14</td>
<td>12.5</td>
<td>20.9</td>
</tr>
<tr>
<td>Palm fatty acids distillate</td>
<td>2.11</td>
<td>2.87</td>
<td>3.64</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>3.43</td>
<td>3.19</td>
<td>2.93</td>
</tr>
<tr>
<td>Salt</td>
<td>3.23</td>
<td>4.48</td>
<td>5.73</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.83</td>
<td>3.48</td>
<td>6.15</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>1.61</td>
<td>2.65</td>
<td>3.68</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>0.0</td>
<td>0.98</td>
<td>1.97</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>1.24</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DM (g/kg of fresh matter)</td>
<td>404</td>
<td>408</td>
<td>400</td>
</tr>
<tr>
<td>Ash</td>
<td>58.1</td>
<td>60.8</td>
<td>63.1</td>
</tr>
<tr>
<td>CP</td>
<td>159</td>
<td>164</td>
<td>171</td>
</tr>
<tr>
<td>Crude fat</td>
<td>36</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>Starch</td>
<td>173</td>
<td>194</td>
<td>223</td>
</tr>
<tr>
<td>NDF</td>
<td>206</td>
<td>278</td>
<td>248</td>
</tr>
<tr>
<td>INDN^4</td>
<td>76.4</td>
<td>78.0</td>
<td>78.2</td>
</tr>
<tr>
<td>DNDF^5</td>
<td>230</td>
<td>200</td>
<td>170</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>1.32</td>
<td>1.25</td>
<td>1.15</td>
</tr>
<tr>
<td>Gross energy^b (MJ/kg of DM)</td>
<td>19.0</td>
<td>19.0</td>
<td>19.1</td>
</tr>
<tr>
<td>NEL₂₀ (MJ/kg of DM)^7</td>
<td>6.57</td>
<td>6.59</td>
<td>6.60</td>
</tr>
<tr>
<td>AAT₂₀ (g/MJ NEL₂₀)^8</td>
<td>16.1</td>
<td>15.3</td>
<td>14.3</td>
</tr>
<tr>
<td>PBV₂₀ (g/kg of DM)^9</td>
<td>13</td>
<td>20</td>
<td>29</td>
</tr>
</tbody>
</table>

^1 Dietary concentrate proportions on a DM basis were 49% (C49), 70% (C70), and 91% (C91).

^2 Ingredient composition of the concentrate mixture per kilogram of diet: C49: 170 g of dried beet pulp, 168 g of rapeseed meal, 146 g of barley, 306 g of dried distillers grain, and 1 kg of a standard concentrate mixture. C70: 138 g of dried beet pulp, 201 g of rapeseed meal, 130 g of barley, 248 g of dried distillers grain, and 1 kg of a standard concentrate mixture. C91: 134 g of rapeseed meal, 200 g of barley, 171 g of a standard concentrate mixture, and 1 kg of a standard concentrate mixture.

^3 To obtain a DM content of approximately 400 g/kg of fresh matter, water was added in the following amounts: 0.25 L/kg DM, 0.75 L/kg DM, and 1.25 L/kg DM for C49, C70, and C91, respectively.

^4 Indigestible NDF.

^5 Digestible NDF.

^6 Calculated according to NorFor (Volden and Nielsen, 2011).

^7 Net energy for lactation calculated for 20 kg of DMI/d (Volden and Nielsen, 2011).

^8 Amino acids absorbed in the small intestine (MP) available for milk production calculated for 20 kg of DMI/d (Volden and Nielsen, 2011).

^9 Protein balance in the rumen calculated for 20 kg of DMI/d (Volden and Larsen, 2011).
15 for the other 2 blocks (8 animals). These samples were pooled and stored at −20°C for further chemical analysis. To determine apparent total-tract digestibility of nutrients, 2 fecal samples (0900 and 1500 h) of 250 mL were collected and pooled for each cow at the time of sampling. Cows were equipped with a RumiWatch halter with noseband sensor (ITIN+HOCH GmbH, Liestal, Switzerland; Zehner et al., 2012, 2017) to determine rumination and eating time, and number of eating and rumination chews. The noseband sensor was attached to a halter and continuously recorded pressure of jaw movements related to eating and rumination at a frequency of 10 Hz. Ruminition behavior is considered as chewing of a bolus and characterized by a steady frequency in jaw movements, and eating behavior is considered as the intake and chewing of feed at unsteady frequency (Zehner et al., 2017). The measurements lasted for 3 d (from the morning of d 12 until the morning of d 15 for 16 cows, and d 19–22 for the remaining 8 cows). Before the measurements, animals were habituated to the halter for 4 to 5 h. The RumiWatch Converter software version V0.7.3.2 (ITIN+HOCH GmbH) was used to convert the raw data into hourly data and summed over the day to obtain daily estimates.

Each cow within a block was allocated to one of 4 open circuit respiration chambers, where the distribution of cows on chambers was balanced for breed, parity, and diet. Gas exchange (methane, carbon dioxide, oxygen, and hydrogen) was measured for the last 3 d (except for 4 cows where measurements were omitted for d 1 due to technical issues) of the experiment based on indirect calorimetry using the system described by Hellwing et al. (2012). Measurements within a chamber lasted 30 s and occurred at a 12.5 min sampling frequency between measurements within a chamber. The feed bins of the chambers were automatically regulated to open 30 min after closing the chambers to enable stabilization of gas concentrations before feeding commenced. Cows were confined to the chambers throughout the measurement period and the chambers remained closed except during the twice daily occasions for feeding, milking, and cleaning (approximately 25 min per occasion). Gas measurements recorded during these events were deleted and replaced by average values of the remaining hours of the day to obtain 24 h in total. The airflow rates were set at approximately 2,000 L/min for Holstein cows and approximately 1,500 L/min for Jersey cows. The respiration chamber system was routinely checked for recovery of gases by infusing a known amount of reference gas into each chamber and measuring recovered gas concentrations. The average recovery rates were breed specific, due to the different airflow rates in the chambers. The acquired recovery rates for Holstein were 99.3 ± 0.65% for methane (based on 14 tests in total; 3 or 4 tests per chamber) and 99.3 ± 0.76% for carbon dioxide (based on 32 tests in total; 8 tests per chamber). For Jersey, applied recovery rates were 98.7 ± 0.57% for methane (based on 12 tests in total; 3 tests per chamber) and 98.8 ± 0.43% for carbon dioxide (based on 22 tests in total; 5 or 6 tests per chamber). These recovery rates were used to correct the gas measurements with. All calculations involving gases were based on standard temperature and pressure (0°C or 273.15 K; 101.325 kPa). Densities of 0.716 g of methane/L, 0.090 g of hydrogen/L, 1.965 g of carbon dioxide/L, and 1.429 g of oxygen/L were used to calculate respective gas emission in grams. An oro-ruminal sampling device (FLORA rumen scoop, Geishauser, Wittibreut, Germany; Geishauser et al., 2012) was used to collect liquid samples from the rumen (<40 mL; Larsen et al., 2020) shortly after cows exited the respiration chambers and before afternoon feeding. Rumen liquid was filtered through 1 layer of cheesecloth and transferred to 4 Eppendorf tubes (1 mL), except for samples intended for microbial analysis, which were not filtered. Samples were stored frozen at −80°C until further analysis. Only molar proportions of VFA are reported, whereas VFA concentrations and pH are omitted due to the risk of saliva contamination of the samples (Larsen et al., 2020).

Milk yield was recorded daily on the last 7 d of the experiment, including the 3 d cows stayed in respiration chambers. Milk was sampled during 4 subsequent milkings during chamber stay and analyzed for protein, lactose, and fat content. Milk composition data were used to calculate ECM for the last 7 d of the experiment. Body weight was recorded at the start of the experiment when cows were moved from the loose-housing research farm to the intensive research facilities, and before and after chamber stay.

**Analytical Methods**

Samples of TMR and feces were freeze-dried before grinding using a 1 mm screen, except for subsamples to determine starch content which were ground at a 0.5-mm screen. Dry matter content was determined by drying at 60°C for 48 h (AOAC International, 2000). Samples were analyzed for ash content by combustion at 525°C for 6 h and nitrogen by the Dumas method (Hansen, 1989) using a Vario MAX CN apparatus (Elementar Analysensysteme GmbH, Hanau, Germany). Crude protein was calculated by multiplying the nitrogen content with the factor 6.25. Crude fat was determined by hydrolysis with hydrochloric acid using a Hydrotherm HT6 apparatus (C. Gerhardt GmbH & Co. KG) followed by Soxhlet extraction using petro-
leum ether with a Soxtherm SOX 416 apparatus (C. Gerhardt GmbH & Co. KG; Stoldt, 1952) at an external laboratory (Eurofins Steins Laboratories, Vejen, Denmark). Samples were also analyzed for NDF and indigestible NDF (INDF) using heat-stable amylase and sodium sulfite (Mertens, 2002) following the Ankom procedure (ANKOM, 2017) and data are presented as ash-free NDF. Before analyzing INDF, TMR samples were first incubated in F57 Ankom bags for 288 h (12 d) in the rumen of 3 dry cows fed a standard ration at maintenance (for the ration description see Brask et al., 2013). Starch was analyzed enzymatically using heat-stable α-amylase and amyloglucosidase and measured as liberated glucose (YSI model 2900 analyzer, YSI Inc.; Kristensen et al., 2007). Titanium dioxide was analyzed spectrophotometrically (Lamba 900, PerkinElmer Inc.) as described by Myers et al. (2004) with an adjustment of the method by adding 15 mL of 30% hydrogen peroxide instead of 10 mL and 5 additional drops before measurement of absorbance. Rumen liquid (4 mL) for VFA analysis was stabilized with 1 mL of 25% metaphosphoric acid (MPA) solution to reach 5% MPA in the stabilized sample and analyzed by gas chromatography according to Kristensen et al. (1996) with some modifications. The VFA concentrations were determined in stabilized ruminal liquid after methanolchloroform extraction using 2-ethylbutyrate as internal standard. The gas chromatograph (Trace 1310, Thermo Scientific) was operated with split/splitless injector at 220°C and a flame ionization detector at 250°C. A 30 m × 0.53 mm × 1 µm HP-FFAP column (Agilent Technologies) was operated with split/splitless injector at 250°C and a flame ionization detector at 250°C. A 30 m × 0.53 mm × 1 µm HP-FFAP column (Agilent Technologies) was used with helium as carrier gas at 0.3405 atm. The oven was programmed to increase from 100 to 200°C at 10°C/min. Contents of protein, lactose monohydrate, and fat in milk were analyzed using an infrared analyzer (Milkoscan MSC4000, Foss Analytical) at Eurofins Steins Laboratories (Vejen, Denmark).

**Bacterial DNA Extraction and Analyses**

To extract DNA from rumen samples, a Nucleospin Soil DNA extraction kit (Machery-Nagel) was used as described by Noel et al. (2019). Amplicon libraries covering the V3-V4 region of the 16S rRNA gene were also prepared according to Noel et al. (2019) using universal primers Bac341F and Bac805R as recommended by Klindworth et al. (2013). Amplicon libraries were sequenced on the Illumina MiSeq (Illumina) using 300 bp paired end reads. Bioinformatics on sequence reads were performed in the QIIME2 pipeline (Qiime2 core 2019.7; Bolyen et al., 2019). First, raw sequence data were demultiplexed and quality filtered using the q2-demux plugin with the following options: forward reads truncated after 266 bases, primers were removed, max ee = 2 and trunc_q = 2. This was followed by denoizing and grouping into amplicon sequence variants (ASV) with DADA2 (Callahan et al., 2016). Representative sequences of ASV were aligned with mafft (q2-alignment; Katoh and Standley, 2013) and used to construct a phylogentic tree with fasttree2 (q2-phylogeny; Price et al., 2010). Samples were rarefied (subsampled without replacement) to 36,806 sequences per sample before α-diversity metrics (ASV richness and Shannon diversity; within sample diversity), β-diversity [weighted UniFrac (Lozupone et al., 2007) and unweighted UniFrac (Lozupone and Knight, 2005); between sample diversity] and principle coordinate analysis were calculated using core diversity metrics (q2-diversity). Group significance on α-diversity metrics were performed using Kruskal-Wallis with pairwise comparisons and Benjamini-Hochberg correction to control for the false discovery rate (presented as q-values). Group significance on β-diversity metrics were performed with PERMANOVA with 999 permutations and pairwise comparisons (q2-diversity).

Taxonomy was assigned using the q2-feature-classifier (Bokuilich et al., 2018) to classify-sklearn naïve Bayes taxonomy classifier trained on the Greengenes 13.8 99% operational taxonomic unit reference sequences (McDonald et al., 2012). Alpha rarefaction was performed at sampling depth 30,000 to determine if the sampling depth was high enough to cover the observed diversity (q2-diversity). Alpha rarefaction graphs indicated sufficient sampling depth to cover the sequence variation (data not shown).

Raw microbiome sequence reads were deposited in the National Center for Biotechnology Information (NCBI) short-read archive database under BioProject ID: PRJNA786778 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA786778). After quality filtering and assigning to ASV, a total of 1,214,636 sequence reads were obtained for 24 rumen samples. The samples had an average of 50,610 reads each (minimum = 36,806; maximum = 69,963), which fell into 6,386 ASV. Principal coordinate plots and α-diversity plots were drawn in R (version 4.0.5, R Core Team, 2019) from distance matrices derived in QIIME2.

**Calculations and Statistical Analyses**

Dry matter intake was calculated as the total amount of DM offered minus the amount of DM in the refusals. The average daily amount of titanium dioxide supplied in the feed was used as a marker to calculate fecal DM flow. Apparent total-tract digestibility of DM, OM, CP, starch, and NDF were calculated from the respective nutrient intake and fecal flow. Gross energy intake (GEI), NE1,20, AA absorbed in the small intestine (AAT,20,
metabolizable protein), protein balance in the rumen (PBV20) were calculated in regard to a standard of 20 kg of DMI/d according to NorFor (Volden and Larsen, 2011; Volden and Nielsen, 2011). Energy-corrected milk yield (3.14 MJ/kg) was calculated as ECM yield (kg/d) = milk yield (kg/d) × [(38.3 × milk fat (g/kg) + 24.2 × milk protein (g/kg) + 15.71 × milk lactose (g/kg) + 20.7)/3.140], where lactose is lactose monohydrate (Sjaunja et al., 1991). Feed-conversion efficiency (FCE) was calculated as kg of ECM/kg of DMI.

All variables were averaged per cow (24 observations in total). For VFA and bacterial community data, one observation of a Holstein receiving diet C49 was deleted (23 observations in total) because of a low feed intake on the last day of gas measurements. Another observation was omitted for digestibility and feeding behavior measurements, resulting in 23 observations in total. This observation belonged to a Jersey receiving diet C49, replacing another cow in the experiment, and was omitted because of an insufficient adaptation to the diet before measurements of feeding behavior and digestibility were taken. For this specific cow, data collected later in the experiment during gas measurements were retained in the analysis, because the adaptation length was considered minimal, but sufficient (i.e., 10 d). Feeding behavior data for another Jersey receiving diet C49 was based on 1 d of observation, due to issues with the RumiWatch halter. Proc MIXED in SAS (version 9.4, SAS Institute Inc.) was used to analyze the data including fixed effects for breed (Holstein and Jersey), diet (C49, C70, and C91), parity (primiparous or multiparous), and the interaction between breed and diet, and a random effect for block (6 levels). Cow was the experimental unit. The model included containment as degrees of freedom method. Another mixed model was made for each breed separately including fixed effects for parity and diet and a random effect for block to obtain linear and quadratic polynomial contrasts across diets within breed (quadratic contrasts are not presented in tables, but significant quadratic contrasts and tendencies are presented in footnotes of the tables). Least squares means are reported in tables. The raw sequence read counts from the ASV abundance table were collapsed at the species taxonomic rank and normalized to the relative abundance counts. Spearman rank correlations between the relative abundance of individual rumen bacterial species and molar proportions of VFA in rumen liquid, hydrogen production, and methane emission were made across breed and diet and presented as a heatmap (Supplemental Figure S1, https://doi.org/10.5281/zenodo.7074417; Olijhoek et al., 2022). The heatmap was created in R using the ggplot package (Warnes et al., 2019). Statistical significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS

Interaction Between Breed and Dietary Concentrate Proportion

Dry matter intake, nutrient intake, and nutrient digestibility during the 2 d of feces collection showed no interaction between breed and diet; however, the apparent total-tract digestibility of NDF tended to decrease linearly for Holstein ($P = 0.06$) and significantly decreased in a linear way for Jersey ($P = 0.04$) with increasing concentrate proportion (Table 2). In addition, apparent total-tract digestibility of DM and OM tended to decrease linearly for Jersey ($P = 0.09$ for both), whereas being unaffected for Holstein. Acetate molar proportion decreased linearly with increased level of concentrate for Holstein ($P = 0.03$) and Jersey ($P = 0.05$), and there was no interaction with breed (Table 3). The interaction was significant for propionate molar proportion ($P = 0.04$). There was a linear ($P < 0.001$) and a quadratic ($P = 0.04$) increase in propionate molar proportion for Holstein ($P < 0.001$), whereas there were no linear or quadratic effects for Jersey. The A:P ratio showed a linear decline across diets only for Holstein ($P = 0.001$), even if there was no interaction between breed and diet. For butyrate, there was a tendency for a linear decrease for Holstein ($P = 0.10$) and a tendency for an interaction between breed and diet ($P = 0.08$). For Holstein a linear decline with increasing concentrate proportion was observed for molar proportions of iso-butyrate ($P = 0.02$), whereas for Jersey there was both a linear ($P < 0.001$) and a quadratic decline ($P = 0.04$) with increased proportion of concentrate, in combination with a tendency toward an interaction ($P = 0.07$). For iso-valerate there was a tendency ($P = 0.07$) for interaction between breed and diet. Additionally, a breed and diet interaction was found for caproate molar proportion ($P = 0.04$), and for Jersey, a linear ($P = 0.01$) and a tendency for a quadratic ($P = 0.09$) decline was observed. Daily methane production (interaction $P$-value and linear effect $P$-value for Holstein are 0.001) and methane intensity of Holstein (interaction $P = 0.03$ and linear effect for Holstein $P = 0.01$) were decreased with increased concentrate proportion, but was unaffected for Jersey. Significant interactions between breed and diet were found for methane yield and methane energy losses in percentage of GEI ($P = 0.03$ and 0.04, respectively; Table 4). Methane yield of Holstein was lowered with 18 and 48%, respectively,
for diet C70 and C91 relative to diet C49, whereas the reductions for Jersey were only 17 and 22% for diet C70 and C91 relative to diet C49, respectively. Linear declines in methane emission (all expressions) and $\text{CH}_4$: $\text{CO}_2$ were found for Holstein together with quadratic effects for methane production ($P = 0.01$) and $\text{CH}_4$: $\text{CO}_2$ ($P = 0.001$) as well as tendencies for methane yield ($P = 0.06$) and methane energy loss percentage of GEI ($P = 0.06$), whereas for methane intensity there was no quadratic effect. For Jersey, the linear declines across diets were observed for methane yield ($P = 0.03$) and methane energy loss percentage of GEI ($P = 0.03$). Hydrogen emission only increased linearly for Holstein ($P = 0.03$). For Jersey, the linear increase was not significant even if hydrogen production increased by 57 and 104% (C70 and C91 vs. C49). For BW, milk yield, milk composition, and FCE there was no interaction between breed and diet, but linear declines in milk fat percentage ($P = 0.06$ for Holstein and $P = 0.08$ for Jersey) and FCE ($P = 0.07$ for Jersey) were observed (Table 5). The total eating and rumination time and chews were unaffected by the interaction term, but linear declines were observed. The total time spent eating or ruminating ($P = 0.02$) and the total number of chews during eating and rumination declined linearly for Holstein ($P = 0.01$) with increasing concentrate proportion, whereas these variables tended to decline linearly for Jersey ($P = 0.09$ and $P = 0.08$, respectively; Table 6). Rumination time and rumination chews (all expressions) decreased linearly across diets for both breeds; however, larger declines in rumination time and rumination chews per kilogram of DMI and NDF intake were observed for Jersey than Holstein (interaction: $P = 0.02$ for rumination time per kilogram of DMI and NDF intake, and $P = 0.01$ and $P = 0.001$ for rumination chews per kilogram of DMI and NDF intake, respectively). Eating time and eating chews (all expressions) were unaffected by the interaction term and no significant linear contrasts were observed. The quadratic contrast across diets was significant for Jersey at rumination time (min/d: $P = 0.02$; per kilogram of DMI: $P = 0.04$; per kilogram of NDF intake: $P = 0.01$) and rumination chews (number per day: $P = 0.01$; number per kilogram of NDF intake: $P = 0.03$). The ASV richness (i.e., the number of unique sequence types) of the bacterial community in rumen liquid tended to be lower for Holstein than for Jersey for diet C91 ($q = 0.06$) and was not different for diet C49 ($q = 0.31$) and C70 ($q = 0.39$; Figure 1). The Shannon diversity (an indicator of evenness and richness in the community structure), was lower for Holstein than for Jersey for diet C70 and C91 ($q = 0.05$ for both), but not for diet C49. The weighted and unweighted UniFrac distances (visualized as principle coordinate analysis plots) show separation according to breed and diet (Figure 2). The difference between the breeds was visually less at the lowest concentrate inclusion level (C49) than at the highest concentrate inclusion level (C91) for both weighted and unweighted UniFrac distances.

**Effect of Breed**

Intakes of DM and nutrients were higher for Holstein than Jersey ($P = 0.001$ for all; Table 2). Apparent total-tract digestibility of DM and nutrients were unaffected by breed, except for a tendency for a lower CP digestibility for Jersey than Holstein ($P = 0.10$). Further, Jersey had higher molar proportions of acetate (Table 3). As a result, the A:P ratio as well as acetate plus butyrate-to-propionate [(A+B):P] ratio were higher for Jersey than Holstein ($P = 0.001$ for both ratios). All other VFA were unaffected by breed. Holstein had a consistently lower methane yield (methane per kilogram of DMI; $P < 0.001$), methane intensity (methane per kilogram of ECM; $P = 0.05$; numerically not lower for diet C49), methane loss as a percentage of GEI ($P < 0.001$), and $\text{CH}_4$: $\text{CO}_2$ ratio than Jersey ($P < 0.001$); however, there was also an interaction between breed and diet caused by a larger dietary effect for Holstein (Table 4). Carbon dioxide production and oxygen consumption were higher for Holstein than Jersey ($P < 0.001$ for both), whereas hydrogen production was unaffected by breed. Holstein had a higher milk yield ($P < 0.001$), tended to have a higher ECM yield ($P = 0.07$), and had a higher milk lactose percentage than Jersey ($P = 0.001$), whereas milk fat and protein percentage were lower for Holstein ($P < 0.001$ for both; Table 5). Holstein had a larger BW than Jersey ($P < 0.001$). Feed-conversion efficiency was also lower for Holstein than for Jersey ($P = 0.01$). Further, the total number of chews during eating and rumination was lower ($P = 0.02$) and the total eating plus rumination time tended to be lower for Jersey than for Holstein ($P = 0.09$; Table 6). Jersey spent more time eating per kilogram of DMI and NDF intake ($P = 0.01$ for both) and had a greater number of eating chews per kilogram of DMI and NDF intake than Holstein ($P = 0.01$ for both); however, daily eating time and daily number of chews were unaffected by breed. In contrast, daily rumination time ($P < 0.001$) and number of rumination chews per day were higher for Holstein than Jersey for all diets ($P < 0.001$) even if there was a tendency for an interaction for both parameters ($P = 0.07$ and $P = 0.06$, respectively).

Representative sequences from the 6,386 ASV were assigned to 23 phyla and 110 genera. The relative
### Table 2. Nutrient intake and apparent total-tract nutrient digestibility of Holstein and Jersey cows fed concentrate at 49 (C49), 70 (C70), or 91% of dietary DM (C91)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Holstein</th>
<th>Jersey</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>C49</td>
<td>C70</td>
<td>C91</td>
</tr>
<tr>
<td>Intake (kg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM(^3)</td>
<td>22.3</td>
<td>24.4</td>
<td>21.9</td>
</tr>
<tr>
<td>OM</td>
<td>21.0</td>
<td>22.9</td>
<td>20.5</td>
</tr>
<tr>
<td>CP</td>
<td>3.54</td>
<td>4.01</td>
<td>3.75</td>
</tr>
<tr>
<td>Starch</td>
<td>3.85</td>
<td>4.73</td>
<td>4.90</td>
</tr>
<tr>
<td>NDF</td>
<td>6.81</td>
<td>6.72</td>
<td>5.55</td>
</tr>
<tr>
<td>Digestibility (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>70.6</td>
<td>70.7</td>
<td>69.2</td>
</tr>
<tr>
<td>OM</td>
<td>71.9</td>
<td>72.0</td>
<td>70.6</td>
</tr>
<tr>
<td>CP</td>
<td>63.7</td>
<td>62.0</td>
<td>62.7</td>
</tr>
<tr>
<td>Starch</td>
<td>98.8</td>
<td>98.7</td>
<td>97.8</td>
</tr>
<tr>
<td>NDF</td>
<td>54.7</td>
<td>51.5</td>
<td>46.6</td>
</tr>
</tbody>
</table>

\(^1\)Based on 23 observations (missing observation is for Jersey receiving diet C49).
\(^2\)Linear contrast for diet within breed. Quadratic contrasts for diet within breed were nonsignificant for all variables.
\(^3\)Dry matter intake during the 2 d of feces collection.

### Table 3. Molar proportions of VFA in rumen liquid of Holstein and Jersey cows fed concentrate at 49 (C49), 70 (C70), or 91% of dietary DM (C91)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Holstein</th>
<th>Jersey</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>C49</td>
<td>C70</td>
<td>C91</td>
</tr>
<tr>
<td>Acetate (mol/100 mol)</td>
<td>60.3</td>
<td>57.7</td>
<td>52.7</td>
</tr>
<tr>
<td>Propionate (mol/100 mol)</td>
<td>22.5</td>
<td>27.1</td>
<td>34.1</td>
</tr>
<tr>
<td>Butyrate (mol/100 mol)</td>
<td>12.9</td>
<td>11.6</td>
<td>9.49</td>
</tr>
<tr>
<td>Valerate (mol/100 mol)</td>
<td>1.66</td>
<td>1.66</td>
<td>2.10</td>
</tr>
<tr>
<td>Iso-butyrate (mol/100 mol)</td>
<td>0.82</td>
<td>0.62</td>
<td>0.35</td>
</tr>
<tr>
<td>Iso-valerate (mol/100 mol)</td>
<td>1.24</td>
<td>1.01</td>
<td>0.76</td>
</tr>
<tr>
<td>Caproate (mol/100 mol)</td>
<td>0.56</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>A:P(^4)</td>
<td>2.70</td>
<td>2.15</td>
<td>1.55</td>
</tr>
<tr>
<td>(A + B):P(^5)</td>
<td>3.27</td>
<td>2.57</td>
<td>1.83</td>
</tr>
</tbody>
</table>

\(^1\)Based on 23 observations (missing observation is for 1 Holstein receiving diet C49).
\(^2\)Linear contrast for diet within breed.
\(^3\)The quadratic contrast across diets was significant for Holstein at propionate molar proportion (\(P = 0.04\)) and for Jersey at iso-butyrate molar proportion (\(P = 0.04\)), and showed a tendency for Jersey at caproate molar proportion (\(P = 0.09\)).
\(^4\)Acetate-to-propionate ratio.
\(^5\)Acetate plus butyrate-to-propionate ratio.
### Table 4. Dry matter intake and gas exchange of Holstein and Jersey cows fed concentrate at 49 (C49), 70 (C70), or 91% of dietary DM (C91)

<table>
<thead>
<tr>
<th>Item</th>
<th>Holstein</th>
<th>Jersey</th>
<th>P-value</th>
<th>Linear for Holstein</th>
<th>Linear for Jersey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C49</td>
<td>C70</td>
<td>C91</td>
<td>C49</td>
<td>C70</td>
</tr>
<tr>
<td>DMI² (kg/d)</td>
<td>22.2</td>
<td>23.9</td>
<td>22.0</td>
<td>16.6</td>
<td>18.7</td>
</tr>
<tr>
<td>DMI¹ (kg/kg of MBW per day)</td>
<td>0.18</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
<td>CH₄ (g/d)</td>
<td>407</td>
<td>364</td>
<td>213</td>
<td>347</td>
<td>327</td>
</tr>
<tr>
<td>CH₄ (g/kg of DMI)</td>
<td>18.5</td>
<td>15.2</td>
<td>9.72</td>
<td>21.0</td>
<td>17.4</td>
</tr>
<tr>
<td>CH₄ (g/kg of ECM)</td>
<td>12.0</td>
<td>10.2</td>
<td>7.26</td>
<td>11.7</td>
<td>10.5</td>
</tr>
<tr>
<td>CH₄ (% of GEI)</td>
<td>5.36</td>
<td>4.40</td>
<td>2.81</td>
<td>6.10</td>
<td>5.04</td>
</tr>
<tr>
<td>H₂ (g/d)</td>
<td>0.94</td>
<td>1.50</td>
<td>2.37</td>
<td>0.95</td>
<td>1.49</td>
</tr>
<tr>
<td>CO₂ (g/d)</td>
<td>14,687</td>
<td>14,825</td>
<td>14,407</td>
<td>11,164</td>
<td>11,642</td>
</tr>
<tr>
<td>CH₄:CO₂ ratio¹</td>
<td>0.028</td>
<td>0.024</td>
<td>0.015</td>
<td>0.031</td>
<td>0.028</td>
</tr>
</tbody>
</table>

¹Linear contrast for diet within breed. Quadratic contrasts for diet within breed were nonsignificant for all variables.

### Table 5. Body weight, milk production and composition, and feed efficiency during chamber stay of Holstein and Jersey cows fed concentrate at 49 (C49), 70 (C70), or 91% of dietary DM (C91)

<table>
<thead>
<tr>
<th>Item</th>
<th>Holstein</th>
<th>Jersey</th>
<th>P-value</th>
<th>Linear for Holstein¹</th>
<th>Linear for Jersey¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C49</td>
<td>C70</td>
<td>C91</td>
<td>C49</td>
<td>C70</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>621</td>
<td>623</td>
<td>648</td>
<td>450</td>
<td>427</td>
</tr>
<tr>
<td>Milk yield (kg/d)</td>
<td>33.7</td>
<td>36.4</td>
<td>35.8</td>
<td>21.9</td>
<td>24.7</td>
</tr>
<tr>
<td>ECM yield (kg/d)</td>
<td>34.2</td>
<td>36.2</td>
<td>29.6</td>
<td>29.8</td>
<td>31.0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.95</td>
<td>3.82</td>
<td>2.49</td>
<td>6.51</td>
<td>5.52</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.64</td>
<td>3.59</td>
<td>3.49</td>
<td>4.27</td>
<td>4.27</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.92</td>
<td>4.90</td>
<td>5.03</td>
<td>4.69</td>
<td>4.84</td>
</tr>
<tr>
<td>FCE² (kg of ECM/kg of DMI)</td>
<td>1.54</td>
<td>1.51</td>
<td>1.35</td>
<td>1.81</td>
<td>1.66</td>
</tr>
</tbody>
</table>

¹Linear contrast for diet within breed. Quadratic contrasts for diet within breed were nonsignificant for all variables.

²FCE = feed-conversion efficiency, using DMI during chamber stay.
Table 6. Feeding behavior of Holstein and Jersey cows fed concentrate at 49 (C49), 70 (C70), or 91% of dietary DM (C91)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Holstein</th>
<th>Jersey</th>
<th>SEM</th>
<th>Breed</th>
<th>Diet</th>
<th>Breed × Diet</th>
<th>Linear for Holstein(^2)</th>
<th>Linear for Jersey(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI(^3) (kg/d)</td>
<td>22.0</td>
<td>24.0</td>
<td>22.4</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.62</td>
</tr>
<tr>
<td>NDF intake(^3) (kg/d)</td>
<td>6.71</td>
<td>6.61</td>
<td>5.68</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.10</td>
</tr>
<tr>
<td>Total eating plus rumination time (min/d)</td>
<td>961</td>
<td>901</td>
<td>783</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Eating time (min/kg of DMI)</td>
<td>411</td>
<td>434</td>
<td>431</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.72</td>
</tr>
<tr>
<td>Eating chews (n/kg of NDF intake)</td>
<td>34,807</td>
<td>31,696</td>
<td>30,000</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Rumination time (min/kg of NDF intake)</td>
<td>5,190</td>
<td>4,835</td>
<td>5,302</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>Rumination chews (n/kg of NDF intake)</td>
<td>37,811</td>
<td>32,569</td>
<td>24,680</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.001(^4)</td>
</tr>
</tbody>
</table>

\(^1\)Based on 23 observations (missing observation is for 1 Jersey receiving diet C49). Data for 1 Jersey receiving diet C49 is based on 1 d of observation.

\(^2\)Linear contrast for diet within breed.

\(^3\)DM and NDF intake during feeding behavior measurements.

\(^4\)The quadratic contrast across diets was significant for Jersey at rumination time (min/d: \(P = 0.02\); per kg of DMI: \(P = 0.04\); per kg of NDF intake: \(P = 0.01\)) and rumination chews (number per day: \(P = 0.01\); number per kg of NDF intake: \(P = 0.03\)).
abundance of dominant species in each treatment group are shown in Figure 3. The most dominant genera (>2% of abundance) for both breeds were Prevotella (17.5%), Succinivibrionaceae (12.6%), Bacteroidales (8.4%), Clostridiales (6.35%), Ruminococcus (5.65%), Lachnospiraceae (3.84%), Ruminococcaceae (3.34%), Succiniclasticum (2.73%), Treponema (2.70%), and Coprococcus (2.59%). The heatmap showing correlations between relative abundances of rumen bacterial species with methane emission, hydrogen production, and molar proportions of VFA across breeds and diets is presented in Supplemental Figure S1. Two clusters in bacterial species are observed: one block of 26 species is positively correlated with hydrogen production and molar proportions of propionate and valerate, whereas being negatively correlated with methane emission and molar proportions of acetate and butyrate. An opposite pattern is observed for a block of 55 different species.

**Effect of Increased Concentrate Proportion**

With increased proportion of concentrate in the diets, the intake of starch increased \((P = 0.01)\) and the intake of NDF tended to decrease \((P = 0.09)\), whereas intake of DM, OM, and CP were unaffected by diet (Table 2). Apparent total-tract digestibility of DM, OM, and NDF decreased with increasing dietary concentrate proportion \((P = 0.02, P = 0.02, \text{ and } P = 0.001, \text{ respectively})\). Digestibility of CP and starch were unaffected by diet. Furthermore, molar proportion of acetate \((P < 0.001)\) decreased and proportion of valerate tended to increase \((P = 0.08)\) with increasing concentrate proportion in the diet. The decrease in acetate proportion is reflected in the reduced A:P ratio and \((A+B):P\) ratio \((P = 0.01\) and \(P = 0.02, \text{ respectively}; Table 3\). For proportions of other VFA (i.e., propionate, caproate, butyrate, iso-butyrate, and iso-valerate; tendencies for the latter 3) there was an interaction between diet and breed. Daily methane emission, methane yield, methane intensity, methane energy loss, and \(\text{CH}_4:\text{CO}_2\) ratio all decreased with increasing proportion of concentrate.
DISCUSSION

Dietary Composition

The diets were formulated using different types and levels of concentrates with diet C70 being intermediate to diet C49 and C91. Across diets, starch content had a large increase, NDF and digestible NDF (DNDF) content a large decrease, CP and fat content a slight increase, AAT20 a slight decrease, whereas DM and NEL20 content remained constant when dietary concentrate level increased. Thus, observed effects of the diets on variables relates to the NDF-to-starch ratio, DNDF, and AAT20 content of diets, rather than energy density or fat content.

Methane Emission

Methane emission expressed on DMI and GEI basis were substantially lowered with increasing dietary concentrate proportion as supported by other studies (Agle et al., 2010; Aguerre et al., 2011; Olijhoek et al., 2018). It is well known that addition of dietary fat can also reduce methane emission. However, in the present experiment increased fat level could only account for a minor part of the 48 and 22% reduction in methane yield found for Holstein and Jersey, respectively, because the increase of 6 g of crude fat/kg of DM in diet C91 compared with C49 is expected to give a decrease in methane yield of only about 2% according to a review by Niu et al. (2018), who found a decrease of around 3.5% per additional 10 g of crude fat per kilogram of DM. The increase in CP level of about 1% of DM is not expected to influence methane emission.

For daily methane emission, there was a clear interaction between breed and diet, because Jersey had the lowest emission on the conventional diet (i.e., C49) compared with Holstein, whereas Holstein had the lowest emission for the diet with extremely high concentrate proportion (i.e., C91) compared with Jersey. Lower daily methane production for Jersey than for Holstein for diets up to 70% of DM concentrate has been reported in previous work on lactating dairy cows (Münger and Kreuzer, 2006; Olijhoek et al., 2018; Ud-din et al., 2020) and dairy heifers (Flay et al., 2019).

in the diet ($P = 0.001$, $P < 0.001$, $P = 0.01$, $P < 0.001$, and $P < 0.001$, respectively; Table 4), but for all of these parameters there was an interaction between diet and breed. Hydrogen emission increased with increased concentrate ($P = 0.01$), whereas carbon dioxide production and oxygen consumption were unaffected by diet. Further, milk yield and milk composition were unaffected by diet, except for milk fat percentage, which was markedly decreased with increasing concentrate proportion ($P = 0.01$; Table 5). Feed-conversion efficiency also declined with increasing dietary concentrate proportion ($P = 0.03$). Total eating plus rumination time and the total number of chews during eating and rumination decreased with increased proportion of concentrate ($P = 0.001$ and $P < 0.001$, respectively; Table 6). Increasing dietary concentrate proportion decreased the ASV richness and Shannon diversity measure ($P < 0.001$ for both; Figure 1). The weighted and unweighted UniFrac distances showed clustering according to diet ($P = 0.001$ for both weighted and unweighted UniFrac; Figure 2).
Despite the interaction between diet and breed for daily methane emission and emission as a percentage of GEI, differences in DMI between breeds explain the consequently lower methane emission per kilogram of DMI and as a percentage of GEI for Holstein than Jersey for all diets, which is supported by Olijhoek et al. (2018), but in contrast with other studies on dairy cattle (Münger and Kreuzer, 2006; Uddin et al., 2020) and dairy heifers (Flay et al., 2019). Further, Holstein cows reduced methane yield to a much larger extent than Jersey cows for diet C91 relative to C49 (48 and 22% for Holstein and Jersey, respectively). The decline in methane yield when concentrate level is increased is in line with our previous studies (Figure 4; R² = 0.95 for each breed; Hellwing and Weisbjerg, 2010; Olijhoek et al., 2018). Olijhoek et al. (2018) reported larger reductions in methane yield and methane energy loss for Holstein cows than Jersey cows for a diet with 32% concentrate relative to 61% concentrate. For a less drastic dietary intervention than in the current study, the Holstein and Jersey cows in the current study responded differently to the increase in dietary concentrate level with respect to rumen VFA profile, rumination, and bacterial community structure, whereas no significant interaction between breed and concentrate level were observed for DMI, nutrient intakes, apparent total-tract digestibility of nutrients, milk yield, FCE, eating time and eating chews, as will be described below.

**Volatile Fatty Acids and Nutrient Digestibility**

Reduction in methane emission is generally attributed to shifts in VFA profile and reduced fiber digestibility in the rumen. Indeed, with increasing concentrate proportion and disregarding breeds, increased propionate and decreased acetate molar proportions were observed in connection with lowered total-tract digestibility of DM, OM, and NDF in the current study and agrees with Agle et al. (2010). Molar proportion of propionate in the rumen increased with increasing concentrate proportion in Holstein, whereas there was no effect in Jersey, and indicates a larger hydrogen consumption facilitated by production of propionate for Holstein cows. Propionate synthesis requires hydrogen, and therefore...
increased propionate synthesis per se will lead to a larger
decrease in methane emission. Nevertheless, there was
a significant linear increase in hydrogen emission for
Holstein with increasing level of concentrate, whereas
for Jersey the increase was not significant, which in-
dicates that there was a larger buildup of hydrogen in
Holsteins on diet C91, because of a reduced capacity of
the altered rumen microbiome for diet C91 to convert
hydrogen into methane in these cows. Moreover, the
lack of a linear increase in hydrogen emission for Jersey
despite high numerical percentage increments, indicates
a high variability and therefore a low power.

Jersey cows had a higher rumen A:P ratio than Hol-
stein cows for all diets. Similar results were found by
Olijhoek et al. (2018), but are in contrast to Uddin et
al. (2020), who did not find differences in VFA profile
between breeds when rumen samples were obtained by
rumenocentesis. The greater acetate molar proportion
might suggest more digestion of NDF in the rumen for
Jersey than for Holstein cows. Even though the appar-
etotal-tract digestibility of NDF did not differ be-
tween breeds, a significant linear decrease was observed
for Jerseys, whereas this was a tendency for Holsteins.
This finding perhaps suggests that Jerseys might re-
spond stronger to increased concentrate proportion
regarding decreased NDF digestibility than Holsteins.

The more pronounced effects of increased level of
concentrate in Holsteins than in Jerseys were also ob-
served for the molar proportions of some other VFA
in addition to propionate (i.e., the interaction between
breed and diet was significant for caproate, and there
were tendencies for butyrate, iso-butyrate, and iso-val-
urate) as well as for α- and β-diversity measures. This
jointly indicates that the rumen of Jersey cows was less
affected by increased concentrate levels than the rumen
of Holstein cows, perhaps related to a better buffering
of organic acids and rumen pH related to more intense
chewing per kilogram DMI during feed ingestion by
Jersey. Another more speculative explanation could be
a more regular feed intake pattern over the day by Jer-
sey cows (Aikman et al., 2008). Both suggestions imply
a lower occurrence of subacute rumen acidosis for Jer-
sy cows than for Holstein cows and could explain why
the rumen bacterial community of Jersey cows was less
affected by increased dietary concentrate levels. Unfor-
nately, rumen pH cannot provide reliable information
on rumen acidosis when using oro-ruminal sampling
devices, because this rumen liquid collection method
increases the risk for significant contamination of the
sample with saliva making pH and concentrations of
organic acids unreliable (Larsen et al., 2020); however,
molar proportions of VFA can be regarded as valid. An
increase in propionate molar proportion for diet C91
relative to C49, reduced A:P ratio, and observed milk
fat depression, indicates that some degree of subacute
rumen acidosis might have occurred in Holstein cows.

Rumen Bacterial Community Structure

The lowered NDF digestibility with increasing di-
etary concentrate level can be attributed to a lower
NDF digestibility for barley straw and concentrate,
which both have a higher proportion of INDF in total
NDF compared with silages and other factors, such as
substrate preference by rumen microbes and inhibition
of fibrolytic bacteria at lower rumen pH. The latter
suggestion can be supported by the trend in lower
abundances of the major fibrolytic bacteria (i.e., Fibro-
bacter succinogenes and Ruminococcus species) as visu-
alized in Figure 3. The bacterial community was evalu-
ated with α- and β-diversity measures. Alpha-diversity

![Figure 4. Methane yield as a function of dietary concentrate proportion for Holstein (closed symbols and solid regression line) and Jersey cows (open symbols and dashed regression line) from the current study (triangles), Hellwing and Weisbjerg (2010; diamonds for Holstein fed early-cut grass silage and squares for Holstein fed late-cut grass silage), and Olijhoek et al. (2018; circles); R² = 0.95 for each breed.](image-url)
is based on the number of features (i.e., ASV richness, closest definition to species) in a sample and additionally the evenness of features in case of the Shannon diversity index, and provides information how diverse the microbial community in a sample is. Beta-diversity shows differences in the microbial community between the treatments. For the weighted UniFrac distances, the relative abundances of ASV are accounted for and therefore mainly show differences in the abundance of dominant species. The unweighted UniFrac distances ignore abundance of species and thereby provide information on the presence and absence of all species. Dietary concentrate level clearly affected the rumen bacterial community as evident by α- and β-diversity measures, which is in accordance with Noel et al. (2019). In other words, the bacterial community was less diverse with increasing dietary concentrate proportion, which can lead to lower methane emission when fibrolytic bacteria become less abundant. Diet has previously been ascribed as the main factor for differences in microbial community structure (Henderson et al., 2015) and is likely a contributing factor for the distinct patterns of associations seen in correlation of bacterial species with phenotypes observed in Supplemental Figure S1. This figure shows that certain bacterial species are associated with increased hydrogen gas production and hydrogen consuming pathways in the rumen, such as propionate and valerate production, whereas other species are associated with increased methane emission and hydrogen producing pathways, such as acetate and butyrate production. The α-diversity of the rumen bacterial community was not significantly different between Holsteins and Jerseys fed diet C49, which is in contrast with Paz et al. (2016), who reported higher richness based on the number of observed operational taxonomic units for Holstein cows than for Jersey cows fed with 49% concentrate of DM. For C91, both α-diversity measures were lower for Holsteins than Jerseys. Both β-diversity measures in the current study were affected by breed, which agrees with Paz et al. (2016) and Noel et al. (2019). More specifically, the abundance of cellulolytic bacteria was reported to differ between breeds (Paz et al., 2016). Overall, these findings indicate that the rumen bacterial community differs in diversity and structure between Holsteins and Jerseys as affected by diet, which can have implications for rumen fermentation. Though, this data set with extreme differences in dietary concentrate proportion gives valuable insight into the relation between specific bacterial species and rumen metabolism, it is beyond the scope of this article to go into detail of specific bacterial species and their functions in rumen fermentation.

Dry Matter Intake and Milk Production

Holstein and Jersey differ in body size, which causes a higher intake of DM and nutrients for Holstein than Jersey. Generally, DMI and ECM yield is increased when dietary concentrate proportion is increased to moderately high levels (Xue et al., 2011; Huhtanen and Hetta, 2012; Olijhoek et al., 2018) followed by a lower rumen fill and increased energy intake. In the current study, DMI and ECM yield were not significantly affected by diet, with diet C91 being substantially higher in concentrate proportion than applied in most other studies on dairy cattle. It is worth noting, that care should be taken when interpreting milk yield data due to the experimental design of the current study. Net energy content was equal between diets and in agreement, Sutton et al. (2003) reported unaffected milk yield when Friesian dairy cows were fed restricted with a diet containing 90% concentrate (barley and soybean meal) compared with 60% concentrate and with similar digestible energy content between diets. Perhaps DMI did not increase in the current study due to rumen imbalance caused by mild rumen acidosis for Holstein cows or the slightly decreasing AAT20 with increasing concentrate proportion. Lowered ruminal pH together with elevated molar proportions of propionate can negatively affect the number of precursors available for lipogenesis in the mammary gland and cause milk fat depression and changes in milk fatty acid profile (Sandri et al., 2020). Other studies also showed a reduction in milk fat content with increasing dietary concentrate proportion (Sutton et al., 2003; Aguerre et al., 2011; Huhtanen and Hetta, 2012).

Feeding Behavior

Measurements of feeding behavior began 12 d after the beginning of experimental feeding (i.e., 8 d after feeding 100% of the experimental diet) for 16 of the 24 cows and 19 d after the beginning for the remaining 8 cows. The adaptation period is considered sufficiently long to rely on the results for rumination and feeding behavior in the present study, because rumination time, feeding time, and feeding rate took up to 4 d to stabilize following a change from a normal lactation diet to an energy-reduced lactation diet (30% of DM dilution with straw) in a recent study by Franchi et al. (2022; see their graphical abstract). A point to consider when interpreting the results of feeding behavior is that the RumiWatch system is a relatively new development. The validation studies involving the RumiWatch Converter version 0.7.3.2 or earlier showed good performance for rumination time when comparing
observations obtained with the RumiWatch system and visually (Kröger et al., 2016; Ruuska et al., 2016; Rombach et al., 2018). However, the number of rumination chews were underestimated (Kröger et al., 2016) and a small overestimation of eating time occurred for cows kept in tiestalls (Ruuska et al., 2016). These findings should be kept in mind when interpreting the data.

Holstein and Jersey responded differently to increased dietary concentrate proportion regarding rumination and chewing, whereas eating was unaffected by the interaction between breed and diet. Jersey had a much larger decrease in rumination time and rumination chews per kilogram of DMI and NDF intake for diet C91 than Holstein. Explanations may be found when detailing the effect of diet and breed separately. All 3 expressions of rumination time and rumination chews were reduced with increasing concentrate proportion (diet effect) and is in accordance with Kröger et al. (2016), who used the RumiWatch system to investigate diets containing 0 and 65% concentrate of dietary DM. Reduced rumination is associated with a reduced physically effective NDF content of high concentrate diets, which also negatively affected rumination and total chewing time (per day, per kilogram of DMI, and per kilogram of NDF intake), but not eating time (Cao et al., 2021). Rumination processes are therefore likely more affected by physical structure of the feed than is ingestion of feed and the decrease in rumination time can negatively affect the rumen environment and nutrient digestibility. Differences between breeds were observed for rumination and eating behavior. Daily rumination time was longer and the daily number of rumination chews were higher for Holstein than Jersey and agrees with Prendiville et al. (2010). Jersey cows also spent more time eating per unit of feed for all diets during feed ingestion than Holstein cows. Aikman et al. (2008) found that lactating Jersey cows had a 36% longer total chewing time per kilogram of BW than Holstein cows when offered TMR, and that Jersey cows spend more time eating per unit of DMI and NDF intake, but also more time for rumination. In the current study, the number of chews during eating per kilogram of DMI and NDF intake was 29% (average across diets) higher for Jersey cows than for Holstein cows, which was associated with a lower daily DMI and NDF intake for Jersey. Prendiville et al. (2010) reported similar findings for grazing Holstein and Jersey cows, which was attributed to the smaller physical size of Jersey cows. Collectively, these findings indicate a higher degree of mastication and efficient particle size reduction of a given diet during ingestion for Jersey cows than Holstein cows, which would lower the necessity for particle size reduction during rumination for Jersey cows (Beauchemin, 2018). Responses in eating and rumination time of Holstein and Jersey to similar diets may profoundly be diet specific (e.g., chemical composition) and could occur when chewing behavior is likely to be affected, such as for diets high in concentrate because of a small particle size. More intense mastication during ingestion and rumination stimulates saliva production, which buffers rumen organic acid production and rumen pH (Beauchemin, 2018). Consequently, rumen digestibility of fiber may improve and passage rate may increase. A higher passage rate of particles from the rumen and hence shorter retention times in the rumen and total-tract for Jersey cows than Holstein cows at similar digestibility of OM or increased digestibility of NDF have been reported previously (Ingvartsen and Weisbjerg, 1993; Aikman et al., 2008) and indicates a higher rate of digestion for Jersey cows. Overall, this efficient digestive process for Jersey cows together with more intense mastication during eating can possibly explain the higher methane emission per unit of DMI compared with Holstein cows facilitated by higher (A + B):P ratio. In general, differential responses in feeding behavior, methane emission, and rumen and digestive processes between Holstein and Jersey cows to similar diets is little explored to date and warrants further investigation.

CONCLUSIONS

Diets containing concentrates up to 91% of dietary DM were effective in lowering enteric methane emission and Holstein cows responded stronger than Jersey cows to increased dietary concentrate level regarding methane mitigation. Therefore, feeding diets high in concentrates are a less effective methane mitigation strategy for Jersey cows than for Holstein cows. In case of shortage in the availability of roughage, diets high in concentrates might be suitable for Jersey cows, because their rumen environment is less affected; however, Jersey cows responded stronger with regard to rumination than Holstein cows. Despite that the diet with concentrates at 91% of DM was a very efficient methane mitigation strategy for Holstein, it cannot be recommended as a long-term strategy due to the risk of rumen acidosis, as indicated by a high propionate molar proportion and low A:P ratio.

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