J. Dairy Sci. 108:8350–8366
https://doi.org/10.3168/jds.2024-26108

Can high-lipid concentrates offset the high enteric methane production caused by high-forage diets fed to lactating dairy cows?

N. Ayanfe,[1]* I. Tapio,[2] S. Ahvenjärvi,[1] M. Rinne,[1] A. Sairanen,[3] and A. R. Bayat[1]
[1]Animal Nutrition, Production Systems, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland
[2]Genomics and Breeding, Production Systems, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland
[3]Animal Nutrition, Production Systems, Natural Resources Institute Finland (Luke), FI-71750 Maaninka, Finland

ABSTRACT

Ruminants have the ability to utilize forages to produce high-quality products for human consumption. However, feeding high-forage diets can increase enteric methane (CH_4) production. Therefore, this study examined the viability of using a high-ether extract concentrate as a compensatory strategy to mitigate elevated CH_4 production, while assessing effects on performance, digestibility, energy utilization, and ruminal and fecal microbiota. Four multiparous Nordic Red dairy cows in early lactation (averaging 61 ± 16.4 DIM) were randomly assigned to a 4 × 4 Latin square design in a 2 × 2 factorial arrangement of dietary treatments. There were four 21-d experimental periods, each consisting of a 16-d adaptation and a 5-d sampling period in metabolic chambers. The diets comprised TMR based on grass silage with a forage-to-concentrate (FC) ratio of 70:30 (high-forage; HF) or 50:50 (low-forage; LF) mixed with either a low-ether extract concentrate (LEE) containing rapeseed meal and barley or a high-ether extract concentrate (HEE) containing rapeseed cake and oats. Ether extract concentrations were 36, 48, 32, and 53 g/kg of DM for HF-LEE, HF-HEE, LF-LEE, and LF-HEE, respectively. The DMI was 3.2 kg/d lower in cows fed HF compared with LF diets, with no effect of concentrate type. Digestibility of NDF and OM was significantly lower in the LF-HEE diet compared with the other diets. Additionally, HF diets reduced milk yield, which further declined with HEE supplementation. The LF and HF diets had similar daily CH_4 production (grams/day), and CH_4 yield (grams/kilogram of DMI) was greater for HF compared with LF diets. Ether extract supplementation was more effective in reducing CH_4 yield in the HF than the LF diet (7.1 vs. 4.2 g CH_4/kg of DMI per each kilogram added ether extract). Methane intensity was the lowest in the LF-HEE diet; however, HF-HEE diet reduced CH_4 intensity (grams/kilogram of ECM) by 5.5% compared with HF-LEE, matching that observed with LF-LEE diet. Significant changes in rumen bacterial, archaeal, and fungal abundances were induced by the dietary FC ratio, and rumen protozoa and fecal fungi abundances were affected more by concentrate type. In conclusion, incorporating HEE in an HF diet reduced CH_4 intensity to levels comparable to those achieved with LEE in LF diets. These findings suggest that supplementing HF diets with HEE concentrates could be a practical and cost-effective strategy for sustainable dairy production.

Key words: dietary manipulation, greenhouse gas, rapeseed cake, oats, microbiota

INTRODUCTION

Dairy production is a vital component of global food security due to the ruminants' ability to convert human-inedible ingredients such as forages and by-products into high-quality dairy and meat products for human consumption. However, ruminants are also significant contributors to climate change through GHG emissions. Livestock contribute 14.5% of global anthropogenic GHG emissions from the agrifood sector (Ripple et al., 2014), with enteric fermentation accounting for 91% of the livestock methane (CH_4) emissions (FAOSTAT, 2021). Besides the climatic consequences of enteric CH_4 emission, it also represents about a 6.0% ± 1.18% loss in gross energy (**GE**) intake of dairy cows (Niu et al., 2018). Reducing CH_4, which is a potent, short-lived, and the second most important anthropogenic GHG, by 34% could potentially limit global warming to 1.5°C by 2030 (IPCC, 2023). Consequently, there has been growing research focused on developing abatement strategies to attain this target (Arndt et al., 2022).

It is well-known that feeding supplementary concentrates can boost milk production and lower CH_4 intensity of dairy production (Arndt et al., 2022). However, its intensified use may have adverse effect on cow's

---

Received December 4, 2024.
Accepted May 9, 2025.
*Corresponding author: nisola.ayanfe@luke.fi

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Table 1. The chemical composition of concentrate ingredients[1]

Item	Barley	Oats	Rapeseed meal	Rapeseed cake[2]
DM, g/kg	860	860	890	910
Composition in DM[3] (g/kg, unless otherwise stated)				
Ash	29	38	80	80
CP	115	127	379	370
EE	22	60	44	120
Starch	610	440	45	—
NDF	210	290	270	—
iNDF	37	115	125	—
ME, MJ/kg of DM	13.2	12.1	11.4	13.1
MP	96	92	169	162

[1] Chemical composition of barley, oats and rapeseed meal extracted from Finnish feed tables (Luke, 2025).

[2] Cold pressed rapeseed cake with low concentrations of glucosinolates and erucic acid (Alavuden Öljynpuristamo Oy, Alavus, Finland).

[3] iNDF = indigestible NDF.

health while also contributing to feed-food competition. Forage production, on the other hand, contributes to climate-friendly farming by enhancing carbon sequestration, biodiversity, and soil structure (Richter et al., 2024). Although the use of grass as feedstock in green biorefinery to provide novel feed and food ingredients is gaining interest (Gaffey et al., 2023), ruminants are still the main utilizers of forages. Increasing forage in diets of dairy cows may improve animal health and promote cost-effectiveness and sustainability in dairy production, but it could also lead to higher enteric CH_4 production (Aguerre et al., 2011).

Dietary fats and oils are known for their potential to reduce enteric CH_4 production (Boadi et al., 2004; Arndt et al., 2022) while modifying the rumen microbiome (Martin et al., 2010). In a study conducted by Bayat et al. (2017), supplementing sunflower oil (50 g/kg of DM) was more effective in reducing enteric CH_4 production with high- rather than low-forage diets (forage-to-concentrate [**FC**] ratio of 65:35 vs. 35:65). In previous studies (Brask et al., 2013; Bayat et al., 2022), rapeseed cake, a by-product of the oil industry, was used as a feed ingredient containing a high amount of residual oil and reduced enteric CH_4 production without negative effects on feed intake. Oats (*Avena sativa*) grain is another natural feed ingredient with relatively high-lipid content (70–90 g/kg of DM) that can be used in combination with rapeseed cake to increase the lipid content of the diet (Räisänen et al., 2024). Aside from reducing CH_4 production, the dietary inclusion of plant oils or oilseeds can enhance feed efficiency and enrich the nutritional value of milk fatty acids (Bayat et al., 2018). Nevertheless, the feasibility of this approach will depend on the relative costs associated with forage and concentrates, as well as profitability in terms of product prices.

This study aimed to find practical solutions for reducing enteric CH_4 production in a high-forage diet through natural feed ingredients. The chosen concentrate components are readily accessible, making the findings practical for dairy farms striving for sustainable production. We hypothesized that high-ether extract concentrates could offset increased CH_4 production from high-forage diets in lactating dairy cows. The objective of the study was to evaluate the effects of lipid inclusion to high-forage diets on enteric CH_4 production, animal performance, ruminal fermentation, nutrient utilization, and ruminal and fecal microbial populations.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

The experiment was conducted at the experimental dairy barn of the Natural Resources Institute Finland (Luke), Jokioinen (60°49′N, 23°28′E) from January to April 2022. Animal management and experimental procedures used in this study received authorization from the National Project Authorization Board (ESA-VI/40540/2021; Finland) according to established guidelines by the European Community Council Directive 2010/63/EU (European Union, 2010). Four multiparous Nordic Red lactating dairy cows were randomly selected for the study. The cows were at (mean ± SD) 61 ± 16.4 DIM, weighing 663 ± 28.9 kg and producing 45.7 ± 2.57 kg of milk per day at the beginning of the experiment. There were 4 experimental periods (21 d each) comprising a 16-d diet adaptation period and a 5-d data and sample collection period. During the adaptation period, the cows were loose-housed, while during the collection period, each cow was housed in a metabolic chamber.

The experimental design was a 4 × 4 Latin square with 2 × 2 factorial arrangements of dietary treatments. The diets were composed of TMR based on grass silage accommodating 2 dietary factors. The first factor was the

Table 2. Formulation of dietary concentrates and TMR, and their chemical composition as well as grass silage composition

Item	Grass silage[2]	Low-EE concentrate	High-EE concentrate	TMR[1]			
				Low forage		High forage	
				Low EE	High EE	Low EE	High EE
Feed ingredient, g/kg DM							
Grass silage	—	—	—	500	500	700	700
Barley grain	—	500	—	250	—	150	—
Oats grain	—	—	500	—	250	—	150
Molasses sugar beet pulp	—	220	220	110	110	66	66
Rapeseed meal[3]	—	250	—	125	—	75	—
Rapeseed cake[4]	—	—	250	—	125	—	75
Vitamin and mineral mixture[5]	—	30	30	15	15	9	9
Composition, g/kg of DM unless otherwise stated							
DM, g/kg as fed	227	875	883	551	555	421	424
OM	938	925	925	931	931	934	934
CP	132	171	171	151	151	144	144
EE	41	24	65	32	53	36	48
Starch	NA[6]	330	265	165	132	99	79
NFC	295	498	428	396	361	356	335
NDF	470	233	262	351	366	399	408
GE, MJ/kg of DM	18.0	18.0	19.0	18.1	18.6	18.1	18.4
ME,[7] MJ/kg of DM	11.8	12.1	12.0	10.6	10.7	10.0	10.4
MP[8]	85.6	112	109	98.8	97.3	93.5	92.6

[1] Total mixed ration consisted of either 50% or 70% forage (low- or high-forage; on a DM basis) supplemented with low- or high-EE concentrates.

[2] Fermentation quality: pH 3.92; in DM, g/kg: lactic acid, 62; formic acid, 15; acetic acid, 21; propionic acid, 0.5; butyric acid, 0.6; ethanol, 15; water-soluble carbohydrates, 17; in vitro cellulase OM digestibility 0.785 g/g of OM; in total N, g/kg: soluble N, 499; ammonium-N, 43.

[3] Solvent extracted rapeseed meal with low concentrations of glucosinolates and erucic acid (Lantmännen Agro Oy, Kouvola, Finland).

[4] Cold pressed rapeseed cake with low concentrations of glucosinolates and erucic acid (Alavuden Öljynpuristamo Oy, Alavus, Finland).

[5] Lypsykivennäinen Tiineys+, Hankkija Oy, Hyvinkää, Finland; Mineral and vitamin composition: Ca, 210 g/kg; P, 15 g/kg; Na, 95 g/kg; Mg, 90 g/kg; Zn 5350 mg/kg; Mn 3180 mg/kg; Cu, 810 mg/kg; I, 180 mg/kg; Co, 20 mg/kg; sodium selenite, 20 mg/kg; selenomethionine 10 mg/kg; Vitamin A, 250,000 IU; Vitamin D_3, 110,000 IU; Vitamin E, 3000 mg/kg; biotin 30 mg/kg.

[6] NA = not analyzed.

[7] Calculated for individual feeds using Finnish feed evaluation system (Luke, 2025) and for diets based on metabolic chamber measurements.

[8] Calculated for both individual feeds and diets using Finnish feed evaluation system (Luke, 2025).

FC ratio, which was 70:30 (high forage; **HF**) or 50:50 (low forage; **LF**) on DM basis. The second factor was concentrate type (**CT**) being either a low-ether extract concentrate (**LEE**), containing rapeseed meal and barley, or a high-ether extract concentrate (**HEE**), containing rapeseed cake and oats, while other feed ingredients were kept similar. Dried barley and oat grains were ground using a roller mill before adding to the concentrates. The experimental grass silage was prepared from primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward, precision chopped to ~3 cm, wilted for 2 h, and preserved in a bunker silo. At harvest, the grass had 202 g/kg of DM content and was treated with a formic and propionic acid–based silage additive (AIV Ässä, Eastman) applied at a rate of 5.0 L/t fresh matter.

The control diet (LF-LEE) was formulated based on Luke (2025) to meet ME and MP requirements of dairy cows producing 40 kg milk/d. The experimental TMR was mixed 3 times weekly using a TMR mixer (Mammut Maschinenbau GmbH) and stored in cool temperature (ca. 10°C) until fed. The cows were fed twice daily at 0700 (1/4 of daily portion) and 1300 h (3/4 of daily portion) during the adaptation period and 4 times per day (0700, 1300, 1700, and 1900 h; equal portions) during the sampling period, allowing for approximately 10% of refusals. The quantity of TMR provided was adjusted individually for each cow considering their previous days' feed intake. The chemical composition of the concentrate ingredients is presented in Table 1, and grass silage, concentrate mixtures, and experimental diets are presented in Table 2.

Data Collection and Sampling

Representative grass silage and concentrate samples were collected twice weekly during the collection period. The silage DM was analyzed using forced-air oven (105°C, 24 h) twice weekly throughout the experiment to maintain the FC ratio of the TMR. Feed samples were stored at −20°C before determining DM concentration and chemical composition. Feed intake was recorded daily by subtracting the amount of feed refusals from the amount of feed given, but only data from the sampling period were used for statistical analysis.

Cows were milked twice daily at 0700 and 1645 h. Milk yields were recorded daily for the entire experimental period, but only measurements between d 17 and 21 were used for statistical analysis. Milk samples were collected during 6 consecutive milkings from d 18 (evening) to d 21 (morning) of each period. The samples were treated with a preservative (bronopol broad spectrum microtabs) and stored at +4°C until infrared analysis of milk fat, CP, and lactose by Valio Ltd. (Seinäjoki, Finland) using MilkoScan 133B (Foss Electric). The composition of milk was calculated based on the average of morning and evening milk yields.

Gas (O_2, CO_2, CH_4, and H_2) exchanges of individual cows were measured using 4 open-circuit respiratory chambers (width × length × height: 288 × 396 × 220 cm) over a 4-d period (d 17 to 21). The first day was designated for the cows to acclimate to the conditions within the chambers. The cows were tied and restrained within the chambers by a neck yoke on a dedicated platform (180 × 126 cm) covered with a rubber mat while having free access to fresh water and a salt block. Gas-exchange measurements in the respiratory chambers are described in detail by Bayat et al. (2022). At the beginning of each measurement, the gas analyzers were calibrated using standard gases (AGA Ltd.). A carbon dioxide recovery test was performed on the chambers after the experiment. A primer gas dose of 114 g of CO_2 was released into each chamber when the air blowers were off to facilitate a faster achievement of steady state conditions and after that CO_2 was released at the rate of 7.5 g/min continuously for at least 75 min when the blowers were active, and the chambers were measuring the gas exchanges. The gas recovery ratio was calculated using data collected after CO_2 concentration became stable and as a ratio between measured and released gases multiplied by 100. The CO_2 recovery was 96.7 ± 3.49% (mean ± SD). To eliminate the potential interference from cow and chamber effects in the statistical model, each cow was kept in the same chamber throughout the different periods of the experiment.

Total excretion of feces and urine was collected over 3 consecutive days, beginning on d 18 at 1000 h. The weight of feces was recorded, and a 5% subsample was stored at −20°C for subsequent chemical analysis. At the end of the experiment, the samples were thawed at room temperature for 1 to 2 d, thoroughly mixed, and a subsample was taken from each animal from each period for chemical analysis. Urine was collected using a lightweight harness and flexible tubing attached to the vulva, connected to plastic containers containing 600 mL of 5 *M* sulfuric acid and they were changed at 12-h intervals. On each sampling day, a urine sample of 0.1% was collected and stored at −20°C for analysis of N and urea concentration. In addition, a 0.2% subsample was collected and stored at −20°C to be freeze-dried for analysis of GE. Precipitations in the urine containers due to interaction with sulfuric acid were collected, stored at −20°C, and dried before N determination.

On d 21 of each experimental period, samples of rumen liquid (500 mL) were collected at 1000 h via the esophagus using the Ruminator sampling device (Profs Products), taking precautions to minimize saliva contamination, to determine rumen-fermentation characteristics and rumen microbiota composition. Immediately after collection, rumen pH was measured using a portable pH meter (VWR International). A 5-mL subsample of rumen liquid was mixed with 0.5 mL of saturated $HgCl_2$ and 2.0 mL of 1 *M* NaOH solutions, chilled on ice, and stored at −20°C for VFA determination. Immediately after preparation, all subsamples were kept on ice before storing at −20°C until analysis for VFA profile by procedures described in Huhtanen et al. (1998). For rumen microbial analysis, rumen liquid samples of 2 mL were snap-frozen in dry ice and stored at −80°C until DNA extraction. For fecal microbial analysis, fecal samples were collected from the rectum on d 19 at 1000 h of each experimental period. The samples were placed into mini-grip bags, snap-frozen in dry ice, and stored at −80°C until DNA extraction.

Chemical Analyses

All analytical procedures for feed, fecal, and urine samples were performed at the Natural Resources Institute Finland (Luke) laboratory (Jokioinen, Finland), following routine laboratory methods according to SFS-EN ISO/IEC 17025:2017 standards, accredited by the Finnish Accreditation Service (T024, Helsinki, Finland). The fermentation profile (lactic acid, formic acid, VFA, ethanol, water-soluble carbohydrates, soluble N, ammonia N, and pH) and in vitro OM digestibility of silages were analyzed using the following procedures. Lactic acid concentration was determined according to Haacker et al. (1983) and formic acid was made according to instructions of the commercial kit (r-Biopharm, Formic acid, UV-method, Roche Diagnostics) with a UV-visible (**vis**) double-beam UV-1800 spectrophotometer (Shimadzu Co.). Determination of VFA was done according to Huhtanen et al. (1998) using an external standardization and HP 6890 gas chromatograph with an automatic injector HP 7683, FID detector, and GC Chemstation Rev.C.01.04 (Agilent OpenLab). Ethanol was analyzed according to manufacturer's instructions through a spectrophotometric method using a commercial kit (Cat. No. 10 176 290 035, Boehringer Mannheim GmbH), and water-soluble carbohydrates were determined using a Shimadzu double-beam UV-vis spectrophotometer UV-1800 according to Somogyi (1945). Soluble N was determined according to the Kjeldahl procedure based on standard method 984.13 (AOAC International, 2019), and ammonia N was analyzed from water extract of samples according to McCullough (1967). In vitro OM digestibility of silages was measured according to the method proposed by Nousiainen et al. (2003), with results corrected using an equation provided by Huhtanen et al. (2006). The DM concentration of feed samples was determined by drying samples at 55°C for 48 h in a forced-air oven (AOAC International, 2019), and silage DM concentration was corrected for volatile compounds losses using equations by Huida et al. (1986). Secondary DM content was analyzed by drying at 105°C for 16 h (AOAC International, 2019). The concentration of NDF was analyzed using an ANKOM 220 fiber analyzer (ANKOM Technology) with Na_2SO_3 according to Van Soest et al. (1991) and results were reported without residual ash. Total N content of feed samples was analyzed by the Dumas method (AOAC International, 2019) using a Leco 628 CHN-elemental analyzer (Leco Corp.; MI 49085) while ether extract (**EE**) was determined based on AOAC International standard method 920.39 (AOAC International, 2019) using an automated extraction unit, Soxtec 8000 (Foss). Ash concentration was determined by igniting the feed samples at 510°C for 16 h in a muffle furnace. Gross energy in silage, concentrates, feces, and urine samples wasmeasured using bomb isoperibol calorimetry (C6010 Bomb, IKA-Werke GmbH & Co. KG), calibrated with benzoic acid (IKA C 723). The starch concentration in concentrates was determined according to manufacturers' recommendations in commercial kits: total starch assay kit K-TSTA/amyloglucosidase/α-amylase method (Neogen-Megazyme) using a UV-vis double-beam UV-1800 spectrophotometer (Shimadzu Co.). Urine N concentration was assessed by the Kjeldahl method using Cu as the digestion catalyst and a Foss Kjeltec 2400 analyzer unit, and DM concentration was measured as described for feed samples. Feces were dried at 60°C, ground through a 1-mm sieve, and analyzed for DM, ash, N, NDF, and EE (after hydrolysis with 3 *M* HCl) using the same procedures as for feed samples.

Microbiota Analyses

Total DNA was extracted from 0.5 mL of rumen liquid and 220 to 270 mg of feces using the DNeasy PowerSoil Pro Kit and following the manufacturer's protocol with initial cell disruption performed by bead beating at 6m/s × 1 min × 3 times in FastPrep (MP Biomedicals). Rumen and fecal bacterial community composition was determined using universal primers 515F and 806R for 16S rRNA gene (Caporaso et al., 2011). The anaerobic fungi community was determined using AGF-LSU-EnVs primers that code the D2 region of the 28S rRNA gene (Young et al., 2022), and rumen ciliate protozoa were determined using P-SSU-316F and GIC758R primers targeting 18S rRNA gene (Ishaq and Wright, 2014). The sequencing libraries were prepared and sequenced at Edinburgh Genetics Ltd. (Penicuik, UK) on an Illumina MiSeq platform. Demultiplexing of sequences was performed by the sequencing provider. Sequence read quality control, which included filtering, denoising, merging, and removal of chimeric reads, as well as clustering of microbiota sequences into amplicon sequence variants (**ASV**), was performed using default settings in DADA2 (Callahan et al., 2016) as implemented in QIIME v. 2 (Bolyen et al., 2019). The ciliate protozoa sequence data were processed as described by Huuki et al. (2022). The sequencing processes resulted in a total of 548,841 quality-filtered reads for rumen bacteria, 275,883 rumen ciliate protozoa, and 134,032 rumen anaerobic fungi, as well as 487,056 reads for fecal bacteria and 734,320 fecal anaerobic fungi. The ASV with less than 10 reads in total or present in only one sample were removed. The Silva 138.1 database (Quast et al., 2013) was used to assign taxonomy to bacteria, and the RIM-DB database (Seedorf et al., 2014) was used for archaea. The ciliate protozoa reference database was obtained from Kittelmann et al. (2015), and the anaerobic fungi AF_LSU database v1.0 was obtained from the Anaerobic Fungi Network (www.anaerobicfungi.org), which was developed based on data previously published by Hanafy et al. (2020). The raw sequence reads are available in the NCBI Sequence Read Archive under BioProject PRJNA1139040 (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1139040).

Calculations and Statistical Analysis

Energy intake and excretion in feces and urine were calculated by multiplying DMI or excreted feces and urine DM contents by their respective GE contents. Apparent total-tract digestibility of each nutrient was calculated as intake minus fecal excretion, divided by the intake. The ME intake was calculated as energy intake minus fecal, urinary, and CH_4 energy, and MP intake was calculated as N intake minus fecal and urinary N excretions. In addition, the ME of diets was calculated as ME intake divided by DM intake, and the MP of diets was calculated using the MP of individual feeds according to the Finnish feed evaluation system (Luke, 2025). Yield of ECM was calculated based on the yields of fat, CP, and lactose (ECM = milk (kg/d) × [38.3 × fat (g/kg) + 24.2 × CP (g/kg) + 16.54 × lactose (g/kg) + 20.7]/3,140) and energy secretion in milk was estimated by multiplying ECM by 3.14 MJ/kg (Sjaunja et al., 1990). Heat produc-

Table 3. Feed and nutrient intakes, and apparent total-tract nutrient digestibility of lactating dairy cows fed low or high-forage diets supplemented with low- or high-oil concentrates[1]

	Low forage		High forage			P-value[2]		
Item	Low EE	High EE	Low EE	High EE	SEM[3]	FC	CT	FC × CT
DM intake, kg/d								
Silage	13.5	14.1	16.9	16.8	0.54	<0.01	0.55	0.49
Concentrates	12.8	13.7	6.9	7.0	0.30	<0.01	0.10	0.18
Total	26.2	27.8	23.8	23.8	0.83	<0.01	0.30	0.34
Nutrient intake, kg/d unless otherwise stated								
OM	24.4	25.9	22.2	22.3	0.78	<0.01	0.30	0.35
CP	3.96	4.20	3.41	3.42	0.124	<0.01	0.30	0.34
NDF	9.3	10.2	9.5	9.8	0.33	0.68	0.084	0.26
EE	0.85^{c}	1.47^{a}	0.86^{c}	1.14^{b}	0.040	<0.01	<0.01	<0.01
Starch	4.22	3.60	2.26	1.88	0.168	<0.01	0.015	0.51
GE, MJ/d	474	516	430	439	15.2	<0.01	0.11	0.25
Digestibility, g/kg unless otherwise stated								
DM	715^{a}	693^{b}	709^{a}	712^{a}	3.9	0.15	0.036	0.011
OM	735^{a}	711^{b}	727^{a}	728^{a}	3.6	0.25	<0.01	<0.01
CP	633	666	621	656	9.6	0.15	<0.01	0.87
NDF	590^{b}	538^{c}	631^{a}	624^{a}	6.7	<0.01	<0.01	0.007
EE	560	679	547	655	16.1	0.24	<0.01	0.70
GE, kJ/MJ	702^{a}	682^{b}	686^{b}	691^{ab}	4.5	0.45	0.16	0.028

[a–c] Treatment means with different letters within a row are significantly different ($P < 0.05$) based on the LSD test. The comparison was done only when the FC × CT interaction was significant.

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

tion of the cows was calculated based on the exchanged oxygen, carbon dioxide, and CH_4 gases, and urinary N excretion was measured in the respiratory chambers using the equation: heat production (kJ/d) = $16.18 \times O_2 + 5.02 \times CO_2 - 5.99 \times \text{urinary N} - 2.17 \times CH_4$, where gas exchanges are expressed as L/d and urinary N as g/d (Brouwer, 1965). The conversion factor 55.24 kJ/g was used to calculate methane energy (Kriss, 1930). Energy balance was calculated by subtracting energy excretion as feces, urine, CH_4, milk, and heat measured in respiratory chambers from the total energy intake. Similarly, N balance was calculated as the difference between N intake and its excretion in feces, urine, and milk.

Obtained data were analyzed by ANOVA using the GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC) with a model including period, FC ratio, CT (i.e., LEE vs. HEE), and interaction of FC ratio and CT as fixed effects, and cow as a random effect. Normality testing was done using the Shapiro–Wilk test and heterogeneity was explored visually. Treatment effects were declared significant at $P < 0.05$ with tendency assumed at $0.05 \leq P < 0.10$ and least squares means were also reported. Pairwise comparisons between treatment means were performed using the LSD test when the interaction of FC ratio and CT was significant.

Rumen and fecal microbiota α diversity were assessed using Shannon and Simpson diversity indexes as well as richness estimate as implemented in the MicrobiotaProcess R package (Xu et al., 2023). To evaluate diet effects on microbiota community structure, between-sample diversity was calculated as Bray–Curtis dissimilarities following Hellinger transformation and visualized using principal coordinate analysis. The significance of FC ratio, CT, and their interaction was evaluated by distance-based permutational multivariate ANOVA (adonis) and defined at the $P < 0.05$ level after 999 permutations, as implemented in the vegan R package (Oksanen et al., 2025). Diet effects on individual microbiota ASV were evaluated using the GLIMMIX procedure as described above. For this analysis, only ASV observed at above 0.01% abundance in at least 50% of samples were included. Before the test, the number of reads was log-base transformed [$\log_2(x + 1)$] and standardized by data centering. For easier interpretation of results, ASV that were significantly affected by treatments were presented in tables as relative abundances (%) within each microbial category. To explore the association between the rumen microbiota and rumen fermentation and feed digestibility characteristics, Spearman correlations were calculated. Only ASV significantly affected by the treatments were included. Significance was defined at $P < 0.05$.

RESULTS

Feed Composition, Intake, and Digestion

The chemical compositions of grass silage, concentrates, and experimental diets are presented in Table 2. The variations in the nutrient composition of experimental diets were influenced by the changing dietary FC ratio as well as the intrinsically differing concentrate ingredients (rapeseed meal and barley vs. rapeseed cake and oats). Rapeseed cake and oats typically contain more lipids than rapeseed meal and barley, with oats also having a higher fiber content than barley. Experimental diets with the same FC ratio had similar CP concentration, but as planned, the EE concentration (average 51 vs. 34 g/kg of DM) was greater for diets with HEE than LEE. Inclusion of HEE resulted in a 65.6% and 33.3% higher EE concentration than LEE in LF and HF diets, respectively. Furthermore, starch concentration was lower in the HF than LF diets (89 vs. 149 g/kg of DM), but an opposite trend was observed for the NDF concentration (404 vs. 359 g/kg of DM). Diets including HEE had higher NDF concentration than those with LEE, primarily due to the replacement of barley with more fibrous oats in HEE. Conversely, diets with LEE had a higher starch content than those with HEE due to the greater starch concentration in barley compared with oats. The HF and LF diets had comparable GE concentration (18.3 vs. 18.4 MJ/kg of DM). However, diets with HEE had slightly higher GE concentration than those with LEE (18.5 vs. 18.1 MJ/kg of DM) due to the increased EE content. The grass silage was characterized by a low pH and ammonia N concentration, indicating good fermentation quality.

Total DMI was lower (11.9%; $P < 0.01$) for cows on HF compared with those on LF diets. Similarly, intakes of OM, CP, starch, and GE were lower ($P < 0.01$) for the HF than LF diets (Table 3). Additionally, feeding HEE led to lower ($P = 0.02$) starch intake and tended ($P = 0.09$) to increase NDF intake. Furthermore, EE intake increased with feeding HEE more profoundly in LF than HF diets (72.9% vs. 32.6%), with the greatest EE intake observed in cows fed the LF-HEE diet compared with the other diets ($P < 0.01$ for FC × CT interaction). Apparent total-tract digestibility of DM, OM, NDF, and GE was affected ($P \leq 0.03$) by the interaction between FC ratio and CT. Supplementing HEE decreased DM, OM, and NDF digestibility in cows fed LF diets but they were unaffected when HEE was incorporated in the HF diets ($P \leq 0.011$ for FC × CT interaction). Dietary FC ratio did not affect CP and EE digestibility whereas their digestibility increased in response to feeding HEE ($P < 0.01$).

Milk Production and Milk Composition

An interaction between FC ratio and CT ($P < 0.01$) was observed for milk yield, where HEE led to a 5.6% decrease in yield in HF diets but resulted in a 13.4% increase in LF diets (Table 4). Feeding HEE increased yields of ECM and milk lactose by 8.9% and 14.7%, respectively, in the LF diets without differing when supplemented in the HF diets ($P < 0.01$ for FC × CT interaction). Milk fat yield tended to increase (by 8.7%; $P = 0.06$ for FC × CT interaction) when HEE was supplemented only in the LF and not in HF diets. Furthermore, milk protein yield was lower (13.5%; $P = 0.035$ for FC × CT interaction) in the HF compared with LF diets. Feeding HF diets increased milk fat concentration by 8.5% but caused a 2.1% decrease in milk lactose concentration compared with LF diets ($P \leq 0.026$), with CT having no effect. Supplementing with HEE reduced milk protein concentration by 8.2% in LF diets, but not in HF diets ($P < 0.01$ for FC × CT interaction). Feeding HEE resulted in a lower feed efficiency expressed as milk/DMI in the HF diet compared with the LF diet ($P = 0.027$ for FC × CT interaction). In contrast, feed efficiency, expressed as ECM/DMI, and efficiency of N utilization for milk production were not affected by either FC ratio or CT ($P \geq 0.16$).

Rumen Fermentation and Methane Production

Rumen pH increased due to HEE supplementation in LF diets, whereas it did not differ in HF diets ($P = 0.015$ for FC × CT interaction; Table 5). High-EE concentrates tended to lower ($P = 0.06$ for FC × CT interaction) total rumen VFA concentration in the LF diet, but not in the HF diets. The acetate molar proportion was greater ($P < 0.01$) in HF compared with the LF diets. Feeding HEE increased the molar proportion of propionate only when included in the LF diet but not in HF diets ($P = 0.031$ for FC × CT interaction). These associations resulted in a higher ($P < 0.01$) acetate-to-propionate ratio in HF compared with LF diets. Butyrate production tended ($P = 0.09$ for FC × CT interaction) to decrease with supplementing HEE in the LF but not in the HF diet.

Daily CH_4 production (g/d) declined ($P < 0.01$) by 6.0% and 8.3% equal to 0.17 and 0.06 g CH_4/g of supplemented EE when HF and LF diets were supplemented with HEE, respectively (Table 6). Methane yield (g/kg of DMI) and CH_4 conversion factor (as % of GE intake) were higher ($P < 0.01$) for cows fed HF compared with those fed LF, and lower ($P < 0.01$) in cows fed HEE compared with LEE. However, when expressed as CH_4 yield per kilogram of supplemented EE, HEE reduced CH_4 yield by 7.1 g/kg in HF compared with 4.2 g/kg CH_4 in LF diets. Feeding HEE lowered CH_4 intensity, as expressed in grams/kilogram of milk, by 17.3% for

Table 4. Milk yield and composition, and feed utilization efficiency of lactating dairy cows fed low or high-forage diets supplemented with low or high-oil concentrates[1]

	Low forage		High forage			*P*-value[2]		
Item	Low EE	High EE	Low EE	High EE	SEM[3]	FC	CT	FC × CT
Yield, kg/d								
Milk	41.7^{b}	47.3^{a}	39.3^{c}	37.1^{d}	0.75	<0.01	0.023	<0.01
ECM[4]	47.2^{b}	51.4^{a}	45.0^{bc}	43.6^{c}	1.82	<0.01	0.087	<0.01
Fat	2.07	2.25	2.03	2.01	0.138	0.019	0.12	0.057
Protein	1.52^{a}	1.58^{a}	1.38^{b}	1.30^{b}	0.026	<0.01	0.77	0.035
Lactose	1.90^{b}	2.18^{a}	1.76^{c}	1.67^{c}	0.040	<0.01	0.021	<0.01
Milk composition, %								
Fat	4.96	4.76	5.16	5.39	0.292	0.012	0.89	0.12
Protein	3.64^{a}	3.34^{c}	3.52^{b}	3.51^{b}	0.056	0.49	<0.01	<0.01
Lactose	4.56	4.61	4.48	4.50	0.075	0.026	0.39	0.62
Total solids	14.2^{a}	13.8^{b}	14.3^{a}	14.5^{a}	0.31	0.017	0.44	0.031
Urea, mg/100mL	13.2	11.3	9.8	8.9	0.72	<0.01	<0.01	0.17
Feed conversion efficiency								
Milk/DMI	1.59^{ab}	1.70^{a}	1.65^{ab}	1.57^{b}	0.039	0.38	0.74	0.027
ECM/DMI	1.80	1.85	1.89	1.83	0.058	0.48	0.92	0.30
N use efficiency (milk N/N intake)	0.38	0.37	0.40	0.38	0.011	0.16	0.17	0.41

[a–d] Treatment means with different letters within a row are significantly different ($P < 0.05$) based on the LSD test. The comparison was done only when the FC × CT interaction was significant.

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

[4] ECM calculated according to Sjaunja et al. (1990).

LF diets ($P < 0.01$ for FC × CT interaction) but did not differ in the HF diets. High-EE concentrate supplementation in both HF and LF diets decreased CH_4 intensity (g/kg of ECM), with the greatest decrease observed with the LF diet ($P \leq 0.015$ for FC × CT interaction). When calculated per kilogram of EE supplementation, the decrease in CH_4 intensity response was 2.7 and 2.5 g/kg ECM for LF and HF, respectively. Methane intensity was similar between the HF diet supplemented with HEE and the LF diet supplemented with LEE (12.0 and 12.3 g/kg of ECM, respectively; $P > 0.05$). Carbon dioxide intensity (g/kg of milk and ECM) was lower ($P \leq 0.031$ for FC × CT interaction) in the LF diet supplemented with HEE compared with other diets. Daily hydrogen production was the highest for the LF diet supplemented with HEE and the lowest for HF diets ($P = 0.027$ for FC × CT interaction).

Energy and Nitrogen Utilization

Intakes of GE and ME were lower ($P < 0.01$) when HF diets were fed compared with LF diets, and HEE tended to increase ($P = 0.09$) ME intake compared with LEE (Table 7). The proportion of GE excreted in feces increased with HEE supplementation in the LF diets, but not in HF diets ($P = 0.028$; for FC × CT interaction). Cows fed HF excreted a higher proportion of GE intake as urine ($P < 0.01$) compared with LF diets, whereas CT had no effect ($P = 0.76$). Milk energy secreted as a proportion of GE intake was unaffected ($P \geq 0.39$) by either FC ratio or CT. A greater proportion of forage in the diet increased ($P \leq 0.015$) the proportion of GE intake lost as CH_4, as well as heat, compared with LF diets, and they decreased ($P \leq 0.038$) with HEE supplementation compared with LEE supplementation. Energy balance was negative for all treatments, but it was lower ($P = 0.017$) in the HF diets compared with LF diets.

There were no interactions between FC ratio and CT for N metabolism ($P \geq 0.41$). Feeding a greater proportion of forage caused a 16.5% decrease ($P < 0.01$) in N intake compared with feeding LF diets, without any effect of CT. High-EE concentrates caused a decrease ($P < 0.01$) in fecal N as a proportion of N intake compared with LEE, and urinary N excretion as a proportion of N intake increased ($P = 0.036$) in the HF diets compared with the LF diets. Negative N balance was observed in all treatments, and it was lower ($P = 0.013$) for HF than LF diets. Nitrogen balance was higher ($P = 0.029$) for cows fed HEE compared with LEE.

Rumen Microbiota

Dietary FC ratio but not CT affected α diversity, where rumen bacteria and anaerobic fungi had significantly higher α diversity estimates in HF compared with LF diets (Shannon $P < 0.05$, Simpson $P = 0.01$). Treat-

Table 5. Rumen-fermentation characteristics of lactating dairy cows fed low- or high-forage diets supplemented with low- or high-oil concentrates[1]

	Low forage		High forage			P-value[2]		
Item	Low EE	High-EE	Low EE	High-EE	SEM[3]	FC	CT	FC × CT
Rumen pH	6.42^{b}	6.63^{a}	6.72^{a}	6.59^{ab}	0.072	0.045	0.45	0.015
Total VFA, mmol/L	124	110	104	110	4.9	0.070	0.38	0.061
Molar proportion, mmol/mol								
Acetate	652	648	678	681	6.1	<0.01	0.96	0.55
Propionate	180^{b}	191^{a}	164^{c}	162^{c}	4.9	<0.01	0.083	0.031
Butyrate	135	125	125	126	2.9	0.16	0.20	0.090
Isobutyrate	5.5^{b}	6.8^{a}	6.6^{a}	6.5^{a}	0.16	0.040	<0.01	<0.01
Isovalerate	5.9^{c}	8.8^{a}	7.7^{ab}	7.2^{bc}	0.61	0.81	0.032	<0.01
Caproate	8.1	7.1	7.0	6.1	0.53	0.090	0.12	0.89
Valerate	13.7	12.8	12.0	10.7	0.53	<0.01	0.048	0.61
Acetate:propionate	3.62	3.41	4.18	4.21	0.144	<0.01	0.32	0.18

[a–c] Treatment means with different letters within a row are significantly different ($P < 0.05$) based on the LSD test. The comparison was done only when the FC × CT interaction was significant.

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

ments had no effect on α diversity estimates for rumen ciliate protozoa (Table 8).

Bray–Curtis dissimilarity analysis demonstrated a significant influence of FC ratio ($P = < 0.001$) on rumen bacterial community structure (Supplemental Figure S1; see Notes; Tapio, 2025). The ASV affiliated with *Bacteroidales* RF16, *Christensenellaceae* R-7, *Lachnospiraceae* NK3A20, *Rikenellaceae* RC9 gut groups, *Muribaculaceae* sp., *Desulfovibrio*, or *Kiritimatiellae* WCHB1–41 were significantly more abundant ($P < 0.05$) in HF diets, but *Succinivibrionaceae* UCG-002, *Acetitomaculum*, and *Succiniclasticum* were more abundant in LF diets. Diversification among dietary preferences was observed among *Prevotella* ASV (Supplemental Table S1; see Notes; Tapio, 2025). In pairwise comparisons, significant community structure differences were observed between LF-LEE and HF-HEE diets (adonis $P = 0.03$). The HF-HEE diet positively affected abundances of *Oscillospiraceae* NK4A214 group, *Lachnospiraceae* NK3A20 group, *Christensenellaceae* R-7 group, [*Eubacterium*] *coprostanoligenes* group, *Gastranaerophilales*, and *Muribaculaceae* sp., but had a negative effect on the abundances of *Mogibacterium*, *Fructilactobacillus*, or *Absconditabacteriales* (SR1) affiliated ASV.

The rumen archaeal community was affected by the dietary FC ratio but not CT. Although ASV affiliated with the *Methanobrevibacer ruminantium* clade, *Methanosphaera* sp. ISO3-F5, *Methanomassiliicoccaceae* Group12 sp. ISO4-H5, and *Methanomassiliicoccaceae* Group 10 were significantly ($P < 0.05$) more abundant in LF diets, 2 *Methanobrevibacter gottschalkii* clade ASV were observed at significantly ($P < 0.05$) higher abundance in HF diets (Supplemental Table S2; see Notes; Tapio, 2025). Concentrate type had a significant effect, more than FC ratio, on the rumen ciliate protozoa community structure (adonis $P < 0.01$; Supplemental Figure S2; see Notes; Tapio, 2025). Several operational taxonomic units (**OTU**) affiliated with *Dasytricha ruminantium*, *Entodinium furca monolobum*, *Epidinium* sp., *Eudiplodinium rostarum*, and *Isotricha prostoma* were significantly ($P < 0.05$) more abundant in HEE diets. The opposite effect was observed for *Entodinium* sp., *Eudiplodinium magii*, *Ostracodinium dentatum*, and *Tripalmaria dogieli* affiliated OTU ($P < 0.05$; Supplemental Table S3; see Notes; Tapio, 2025). Dietary FC ratio had a significant influence on the fungal community structure (adonis $P = 0.024$; Supplemental Figure S3; see Notes; Tapio, 2025). Among individual ASV, *Neocallimastix* and SK3 were significantly more enriched in the LF diet, and *Piromyces* and *Orpinomyces* were more abundant in the HF diet (Supplemental Table S4; see Notes; Tapio, 2025). When shifting from LF-LEE to HF-HEE treatment, changes in rumen microbiota composition were also associated with changes in rumen-fermentation and feed digestibility characteristics (Figures 1 and 2). An increase in acetate was positively associated with several ASV from *Christensenellaceae* R−7, *Oscillospiraceae* NK4A214, *Lachnospiraceae* NK3A20, and *Rikenellaceae* RC9 gut groups, but showed negative correlation with *Dasytricha* and *Epidinium*. A decrease in propionate concentration was negatively correlated with ASV from *Lachnospiraceae* NK3A20 and *Oscillospiraceae* NK4A214 groups, *Muribaculaceae* sp., *Kiritimatiellae* WCHB1−41, *Diplodinium*, *Eudiplodinium*, and SK3, but positive correlation was observed with *Succinivibrionaceae* UCG-002, *Methanobrevibacter gottschalkii* clade, *Epidinium*, and

Table 6. Enteric methane, total carbon dioxide, and hydrogen emissions of lactating dairy cows fed low- or high-forage diets supplemented with low- or high-oil concentrates[1]

	Low forage		High forage			*P*-value[2]		
Item	Low EE	High EE	Low EE	High EE	SEM[3]	FC	CT	FC × CT
Methane								
g/d	579	544	569	522	31.7	0.12	<0.01	0.52
g/kg of DM intake	22.1	19.5	23.9	21.9	0.88	<0.01	<0.01	0.52
g/kg of Milk	13.9^{a}	11.5^{b}	14.5^{a}	14.0^{a}	0.62	<0.01	<0.01	<0.01
g/kg of ECM	12.3^{ab}	10.6^{c}	12.7^{a}	12.0^{b}	0.50	<0.01	<0.01	0.015
% of GE[4] intake	6.75	5.81	7.29	6.56	0.266	<0.01	<0.01	0.38
CO_2								
g/d	16,574	16,528	15,772	15,196	701.0	0.014	0.36	0.43
g/kg of DM intake	632	593	663	637	16.9	0.014	0.028	0.60
g/kg of Milk	398^{a}	349^{b}	401^{a}	409^{a}	12.9	<0.01	0.048	0.015
g/kg of ECM	352^{a}	321^{b}	351^{a}	349^{a}	11.0	0.036	0.019	0.031
Hydrogen, g/d	0.79^{b}	0.91^{a}	0.42^{c}	0.42^{c}	0.064	<0.01	0.021	0.027

[a–c] Treatment means with different letters within a row are significantly different ($P < 0.05$) based on the LSD test. The comparison was done only when the FC × CT interaction was significant.

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

[4] GE = gross energy.

Neocallimastix. Neutral detergent fiber digestibility was positively associated with the *Christensenellaceae* R−7 group, *Prevotella*, *Diplodinium dentatum*, and *Eudiplodinium* sp., and EE digestibility was positively associated with *Enterorhabdus*, *Gastranaerophilales*, *Oscillospiraceae* NK4A214, and *Rikenellaceae* RC9 gut groups, *Diplodinium dentatum*, and SK3 (Figures 1 and 2).

Fecal Microbiota

Dietary treatments had no effect on α diversity of fecal bacteria or anaerobic fungi (Table 8). The dietary FC ratio had a significant effect (adonis $P = 0.026$) on fecal bacterial community structure. From the *Bacteroidota* phylum several ASV affiliated with *Bacteroides*, *Bacteroidales* RF16 group, *Muribaculaceae* sp., *Prevotella*, *Prevotellaceae* (UCG-001, UCG-003, UCG-004), and *Alistipes* were observed at higher abundance in LF diets, and from the *Firmicutes* phylum, ASV affiliated with *Christensenellaceae* R-7, *Lachnospiraceae* NK3A20 groups, and *Oscillospirales* UCG-010 were more abundant in HF diets (Supplemental Table S5; see Notes; Tapio, 2025). Fecal *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* clades were predominant, with *Methanosphaera* ISO3-F5 and *Methanocorpusculum* observed at lower abundances, but archaea were not affected by dietary treatments (Supplemental Table S6; see Notes; Tapio, 2025). Fecal anaerobic fungi were composed of the same genera as those in the rumen, with SK3 and *Cecomyces* being predominant. Several ASV affiliated with SK3 and *Piromyces* were enriched by feeding LEE concentrates, and *Neocallimastix* was detected at higher abundance in the LF-HEE diet (Supplemental Table S7; see Notes; Tapio, 2025). We explored fecal bacteria as a proxy for association with enteric CH_4 production. A negative Spearman correlation was detected with low abundance *Akkermansia*, *Rikenellaceae dgA-11* gut group, *Monoglobus*, and *Kiritimatiellae* WCHB1–41 ASV, and positive correlations were observed with *Bacteroides* sp. and *Prevotellaceae* (UCG-001, UCG-003).

DISCUSSION

Our goal was to lower enteric CH_4 production in high-forage diets (70% on a DM basis) by increasing dietary EE content in the concentrate with little or no negative effects on feed intake and animal performance. To achieve this, we replaced 2 concentrate ingredients with low lipid concentration (i.e., barley and rapeseed meal) with ingredients having high-EE content (i.e., oats and rapeseed cake). However, in addition to changes in the diet EE content, the dietary modification resulted in alterations in concentrations of other nutrients such as NDF, GE, and starch. The LF diet with LEE served as the control, reflecting typical feed rations used, for example, in Northern Europe.

Nutrient Intake and Digestion

In the current study, the lower DMI (11.9%) in cows fed HF diets compared with LF diets was similar to

Table 7. Energy and nitrogen metabolism of lactating dairy cows fed low- or high-forage diets supplemented with low- or high-oil concentrates[1]

	Low forage		High forage			*P*-value[2]		
Item	Low EE	High EE	Low EE	High EE	SEM[3]	FC	CT	FC × CT
Energy metabolism								
GE[4] intake, MJ/d	474	516	430	439	15.2	<0.01	0.11	0.25
ME intake, MJ/d	278	299	237	247	7.6	<0.01	0.086	0.45
Proportion of energy intake, kJ/MJ								
Feces	298[b]	318[a]	314[a]	309[ab]	4.5	0.45	0.16	0.028
Urine	48.0	45.8	61.2	62.3	2.40	<0.01	0.76	0.41
Methane	67.5	58.1	72.9	65.6	2.66	<0.01	<0.01	0.38
Milk	313	313	328	312	10.0	0.41	0.39	0.39
Heat	309	289	325	313	8.3	0.015	0.038	0.53
Milk energy/ME intake, kJ/MJ	534	541	595	555	19.7	0.048	0.32	0.17
Energy balance, MJ/d	−16.7	−12.4	−43.6	−27.6	9.18	0.017	0.17	0.40
Nitrogen metabolism								
N intake, g/d	633	672	545	547	19.8	<0.01	0.30	0.34
MP, g/d	1,330	1,510	1,020	1,100	61	<0.01	0.057	0.41
Proportion of N intake, g/kg								
Fces	367	334	379	344	9.6	0.15	<0.01	0.87
Ureine	297	304	323	333	14.1	0.036	0.45	0.88
Milk	375	369	399	376	11.1	0.16	0.17	0.41
N balance, g/d	−24.8	−5.0	−54.0	−29.0	8.66	0.013	0.029	0.77
N balance, % of N intake	−3.9	−0.7	−10.1	−5.3	1.44	<0.01	0.021	0.60

[a,b] Treatment means with different letters within a row are significantly different ($P < 0.05$) based on the LSD test. The comparison was done only when the FC × CT interaction was significant.

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

[4] GE = gross energy.

the results of Ahvenjärvi et al. (2024), who found an 11.3% decrease in DMI in cows with a similar change in FC ratio. The average substitution rate (kilogram decrease in silage DM intake per kilogram increase in concentrate DM intake) was 0.48, which is well in line with a value of 0.47 derived from a meta-analysis by Huhtanen et al. (2008). The substitution rate was slightly higher on LEE than HEE diets (0.58 vs. 0.40). The lack of an EE supplementation effect on feed intake can be explained by the relatively low levels of supplemented EE content (21 and 12 g/kg of DM for LF and HF diets supplemented with HEE, respectively). The higher fiber digestibility for HF diets is related to lower DMI and longer ruminal mean retention time of forage particles (Allen, 2000; Huhtanen et al., 2009), which affect rumen pH and stimulate higher α diversity of rumen bacteria and anaerobic fungi, as observed in our study. Anaerobic fungi possess a large repertoire of carbohydrate-active enzymes that enable physical and enzymatic degradation of lignin-rich grasses. A majority of the bacteria enriched in HF diets in this study belong to the most prevalent rumen bacterial group (Henderson et al., 2015), but their enrichment has been observed in high-forage diets (Zhang et al., 2017; Ahvenjärvi et al., 2024), suggesting their role in fiber digestion.

Generally, the effects of unsaturated lipids on nutrient digestibility can differ depending on the level of lipid supplementation, the type of forage (grass/legume or cereal) and the dietary FC ratio (Benchaar et al., 2012). The lower fiber and OM digestibility in the LF diet supplemented with HEE (8.8% and 3.3%, respectively) is in agreement with Bayat et al. (2017) reporting 12.6% and 4.6% reduction in apparent total-tract NDF and OM digestibility in LF diets supplemented with sunflower oil. Interestingly, EE supplementation did not affect OM and NDF digestibility in the HF diet, which is consistent with their findings and might be due to lower EE supplementation with the HF-HEE diet. Furthermore, oats, which generally have a higher indigestible NDF concentration compared with barley (110 vs. 37 g/kg of DM; Luke, 2025) may also explain the lower fiber digestibility observed in LF diets supplemented with HEE, likely due to a tendency of increased fiber content and higher indigestible NDF content of oats.

Ruminal Fermentation and Methane Production

Rumen pH being the lowest with the LF diet supplemented with LEE was consistent with higher starch intake and higher total VFA concentration in the rumen

Table 8. Rumen and fecal microbiota α diversity of lactating dairy cows fed low- or high-forage diets supplemented with low- or high-oil concentrates[1]

	Low forage		High forage			*P*-value[2]		
Item	Low EE	High EE	Low EE	High EE	SEM[3]	FC	CT	FC × CT
Rumen bacteria								
Richness	484	449	545	467	30.8	0.23	0.10	0.50
Shannon index	5.31	5.20	5.51	5.39	0.086	0.045	0.21	0.95
Simpson index	0.987	0.984	0.991	0.991	0.0016	0.010	0.17	0.33
Rumen ciliate protozoa								
Richness	198	189	180	183	11.7	0.34	0.77	0.61
Shannon index	3.21	3.21	3.19	3.25	0.084	0.92	0.70	0.68
Simpson index	0.910	0.921	0.923	0.910	0.0074	0.34	0.50	0.40
Rumen fungi								
Richness	26.0	25.7	29.5	28.7	1.01	0.017	0.63	0.81
Shannon index	2.27	2.24	2.56	2.56	0.109	0.011	0.85	0.87
Simpson index	0.787	0.783	0.839	0.851	0.0253	0.016	0.83	0.67
Fecal bacteria								
Richness	346	386	380	370	19.3	0.56	0.35	0.15
Shannon index	5.30	5.44	5.39	5.39	0.054	0.65	0.17	0.14
Simpson index	0.992	0.993	0.993	0.993	0.0004	0.96	0.12	0.10
Fecal fungi								
Richness	26.2	32.2	26.5	27.2	2.62	0.39	0.23	0.34
Shannon index	2.55	2.66	2.66	2.64	0.072	0.43	0.51	0.31
Simpson index	0.890	0.893	0.898	0.884	0.0136	0.92	0.48	0.25

[1] Total mixed rations consisted of either 500 or 700 g forage/kg of DM (low or high forage, respectively) supplemented with low- or high-EE concentrates (24 vs. 65 g EE/kg of DM).

[2] FC = effects of forage-to-concentrate ratio; CT = effects of concentrate type; FC × CT = interaction between forage-to-concentrate ratio and concentrate type.

[3] SEM for n = 4.

compared with other diets. Starch is rapidly fermented by rumen microbes, supporting more propionate production in the rumen. The proportions of ruminal VFA influence CH_4 production, such that propionate formation utilizes H^+, while acetate and butyrate produce H^+, modulating rumen methanogenesis (Martin et al., 2010). The lack of difference in daily CH_4 production between HF and LF diets is a result of lower feed intake and higher CH_4 yield associated with the HF diets. Feed intake is the main driver of CH_4 production (Niu et al., 2018). However, the higher CH_4 yield with the HF diet is consistent with a higher ruminal acetate-to-propionate ratio and higher fiber digestibility.

In the current study, the reduction in CH_4 yield and intensity with HEE supplementation, particularly in LF-HEE treatment, may be due to lower OM digestibility and the high indigestible NDF content from oats. Despite this, the type of oats plays a significant role in CH_4 reduction potential because dehulled oats diminish CH_4 mitigation benefits by increasing digestibility and reducing fiber content (Fant et al., 2021). In the current study, HEE was more effective in reducing CH_4 yield per each unit of added EE in the HF compared with the LF diet (7.1 vs. 4.2 g CH_4/kg of DMI for each kilogram of supplemented EE), supporting our hypothesis. Bayat et al. (2017) showed that the CH_4 yield mitigating effect of sunflower oil supplementation was more pronounced in high- versus low- forage diets (7.3 vs. 1.2 g of CH_4 yield reduction for every kilogram of added oil). The findings of the current study suggest that modifying diets through natural feed ingredients to increase their EE contents rather than adding liquid oil can be an effective and economical strategy in mitigating CH_4.

When CH_4 intensity was expressed as grams per kilogram of ECM, HEE decreased CH_4 intensity by 13.8% in LF and 5.5% in HF diets. This reduction was slightly greater than reported by Räisänen et al. (2024) using similar dietary high-lipid materials, that is, rapeseed cake and oats (11.7% vs. 13.8% in the current study). This was most likely due to higher EE concentrations in the current study and the absence of feed intake depression. However, the inclusion of HEE successfully reduced CH_4 intensity (g/kg of milk or ECM) in the HF-HEE diet to levels comparable to those in the LF-LEE diet, aligning with our hypothesis.

Addition of lipids to the diet is one of the feeding strategies for reducing enteric CH_4 production (Grainger and Beauchemin, 2011) because lipids can lower OM degradation in the rumen and alter microbial activity and biohydrogenation of unsaturated fatty acids (Brask et al., 2013; Bayat et al., 2018). In in vitro cultures it has been demonstrated that different fats or oils may have an inhibitory or no negative effect on bacteria (Enjalbert et al., 2017). In this study, rumen bacteria and archaea

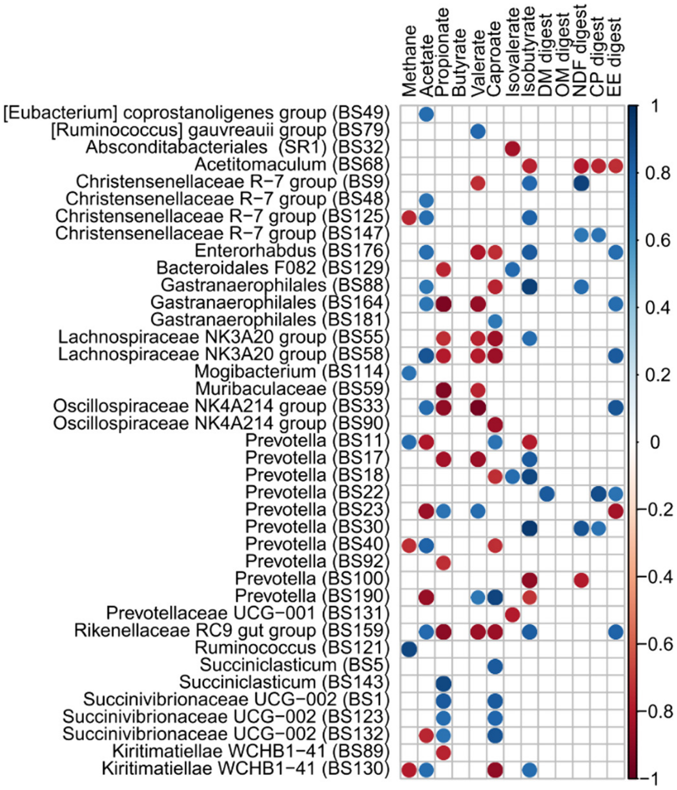

Figure 1. Spearman correlations between rumen bacteria (ASV ID) and rumen-fermentation and nutrient digestibility parameters of cows changing from low-forage, low-ether extract concentrate (LF-LEE) to high-forage, high-ether extract concentrate (HF-HEE) treatment. Only significant ($P < 0.05$) results are included in the figure. Blue indicates a positive association, and red indicates a negative association. Methane (L/d), VFA (mmol/mol), digestibility (g/kg).

did not show strong response to increased EE content, but rumen ciliate protozoa were affected. Although *Epidinium* and *Dasytricha* abundances were higher in diets with LEE supplement, *Entodinium* and *Eudiplodinium-Eremoplastron* genera were more prevalent in HEE diets. It is suggested that smaller ciliate protozoa and their associated archaea might be more active in methanogenesis than larger species. Therefore, taxonomical abundance changes in protozoa could lead to differences in H^+ availability and utilization.

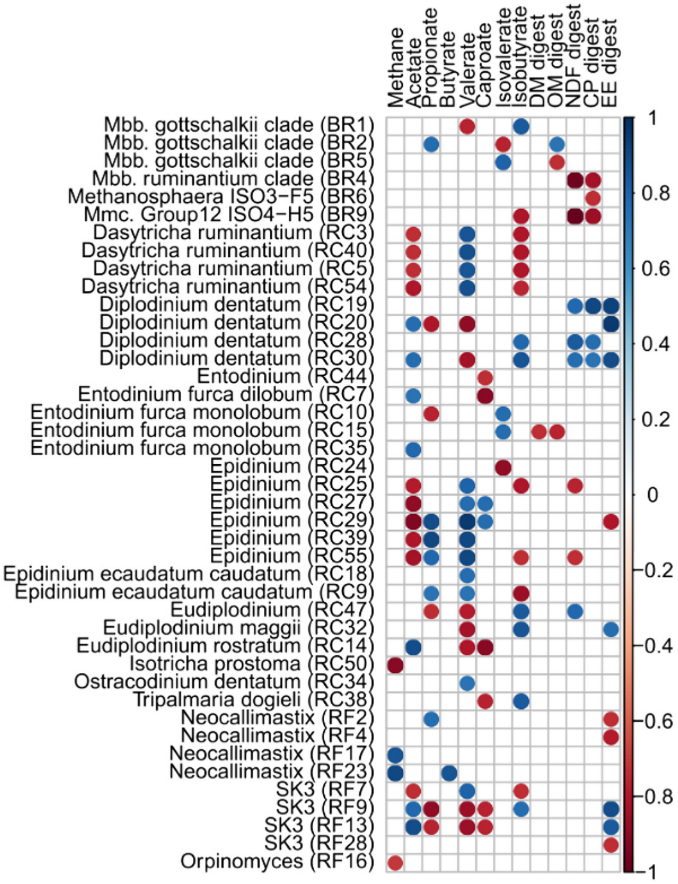

Figure 2. Spearman correlations between rumen archaea, ciliate protozoa, anaerobic fungi (ASV ID) and rumen-fermentation and nutrient digestibility parameters of cows changing from low-forage, low-ether extract (LF-LEE) to high-forage, high-ether extract concentrate (HF-HEE) treatment. Only significant ($P < 0.05$) results are included in the figure. Blue indicates a positive association, and red indicates a negative association. Methane (L/d), VFA (mmol/mol), digestibility (g/kg).

Lactation Performance

Energy intake, especially in the form of glucogenic compounds as the precursors of lactose synthesis, is a critical factor in determining milk volume, which is often affected by dietary forage proportion and lipid content (Weiss and Pinos-Rodríguez, 2009). Therefore, cows consuming HF diets produced less milk compared with the LF cows, which could be explained by the lower intake of feed, ME, MP, and other nutrients needed for milk production. This pattern was also observed in the decreased yields of milk protein, fat, and lactose in HF diets. Our findings agree with other studies that fed high levels of forage to dairy cows (Saliba et al., 2014; Jaakamo et al., 2019; Razzaghi et al., 2022).

Previous studies have shown that replacing rapeseed meal with rapeseed cake (Bayat et al., 2022) or barley with oats (Fant et al., 2021) could positively affect milk yield. In the current study, HEE supplementation increased milk yield for the LF diet (+ 5.6 kg/d) but decreased it for the HF diet (−2.2 kg/d) compared with LEE. This could be attributed to the greater increase in dietary MP, EE, and ME intakes with HEE supplementation (increase of 180 vs. 80 g/d, 0.62 vs. 0.28 kg/d, and 21 vs. 10 MJ/d, respectively) in LF versus HF diets. Supporting this finding, Razzaghi et al. (2022) found that when a low- versus a high-forage diet (FC ratio of 35:65) was supplemented with oil the milk yield increased, which they attributed to the availability of more glucogenic and lipogenic nutrients without affecting DMI. Achieving an optimal balance between glucogenic and lipogenic nutrients is essential for maximizing milk production (Drackley, 1999). The lack of such increases in milk and ECM yields when the HF diet was supplemented with HEE may have stemmed from more lipogenic but less glucogenic substrates (lower starch content in oats), causing an imbalance between them as a result of the dietary modifications. However, it is important to interpret the production responses in the current study with caution due to the small sample size and short duration of the experiment.

A reduction in ruminal pH, coupled with increased propionate and decreased acetate concentrations, can limit precursors needed for lipogenesis in the mammary gland, resulting in a decrease in milk fat synthesis (Sandri et al., 2020). The higher milk fat concentration observed in HF compared with LF diets in the current experiment aligns with previous studies (Aguerre et al., 2011; Saliba et al., 2014). The higher milk volume despite the lower milk fat concentration in LF diets resulted in higher milk fat yield compared with HF diets, which is consistent with the findings of Saliba et al. (2014). Our findings of reduced milk protein yield in HF diets could be the result of the lower diet CP and starch intakes and milk production. In contrast, significant reduction in milk protein concentration in LF but not HF diets when receiving HEE supplementation seem to be a dilution effect due to higher milk production with this treatment, and this is supported by the lack of change in milk protein yield.

The similar efficiencies of milk production (ECM/DMI) observed in the current study for all diets can be interpreted as the effort of cows to maintain the same efficiency for milk production despite energy metabolism being affected by the dietary treatments (i.e., higher urine, CH_4, and heat energy as a proportion on energy intake with HF compared with LF diets and lower proportional CH_4 and heat energy with HEE compared with LEE). This has happened with more energy (and N) mobilization from tissues with HF compared with LF diets, as confirmed by lower energy (and N) balance to compensate for the energy (and N) deficiency with such diets. These results are consistent with those of Razzaghi et al. (2022), who observed the lack of effect from an even more extreme FC ratio and rapeseed oil supplementation level on milk production efficiency as ECM/DMI. In contrast, Räisänen et al. (2024) reported an improvement in feed efficiency when barley and rapeseed meal were replaced with rapeseed cake and oats. Additionally, N use efficiency was also unaffected by the diets, which is consistent with the lack of treatments effect on ECM/DMI.

Energy Utilization

The negative energy balance for all treatments may imply that the energy needs of the cows were not met despite the efforts to balance the diets to meet their ME requirements. The lower energy balance for cows fed HF compared with those fed LF diets is likely due to the reduced ME intake when FC ratio was higher. Contrary to our findings, Razzaghi et al. (2022) reported no effect of changing the FC ratio on energy balance despite lower GE intake in the HF diets. As expected, increasing the forage proportion in the diet resulted in higher CH_4 energy excretion as a proportion of energy intake (from 6.28% to 6.93%), in line with Bayat et al. (2017) and Razzaghi et al. (2022), and this could be attributed to the high OM and NDF digestibility in HF diets compared with LF diets. Both studies also found a decrease in CH_4 energy excretion as a proportion of energy intake with the supplementation of sunflower or rapeseed oil, respectively. In our experiment, a positive effect of feeding HEE was observed, with a 6.6% and 8.3% decrease in CH_4 energy loss as a proportion of energy intake per 10 g/kg of additional fat in LF and HF diets, respectively. In agreement with the current study, Razzaghi et al. (2022) also found 2.7% and 3.0% decreases in CH_4 energy excretion as a proportion of energy intake per 10 g/kg of supplemented rapeseed oil with LF and HF diets, respectively. The higher mitigation response observed in the current experiment could be the result of the lower level of lipid supplementation compared with Razzaghi et al. (2022). This might indicate that the biological response to EE supplementation is stronger at lower levels.

Fecal Microbiota

We explored fecal microbiota as proxy for association with enteric CH_4 production. Although it has been demonstrated that fecal microbiota is not a direct substitute for rumen microbiota, here we show that dietary treatments have affected fecal microbiota abundances, and Spearman correlations identified several bacterial ASV associated with CH_4 production. The low number of animals in this study does not warrant conclusions but suggests that in future studies fecal microbiota should be better explored.

CONCLUSIONS

Adding EE through natural feed ingredients effectively reduced CH_4 production in both low- and high-forage diets. However, when presented per kilogram of added EE, the effect on reducing CH_4 yield was greater in HF versus LF diets (7.1 vs. 4.2 g CH_4/kg of DMI per each kilogram added EE), resulting in similar CH_4

yield and intensity between LF, low-EE and HF, high-EE diets. High-EE concentrates enhanced milk production in the LF diet but decreased it in the HF diet, potentially due to lower starch intake. Rumen bacterial, archaeal, and fungal communities were more affected by dietary FC ratio, and rumen protozoa and fecal fungi were more affected by CT.

NOTES

This work was conducted as part of HiGrass project funded by the Finnish Ministry of Agriculture and Forestry/Makera (Helsinki, Finland; VN/7898/2020). Microbiota-related work was funded from the European Union's (Brussels, Belgium) Horizon 2020 Research and Innovation Programme under grant agreement no. 101000213 (HoloRuminant). We sincerely appreciate the personal research grant awarded to Nisola Ayanfe by the Finnish Association of Academic Agronomists through the Oiva Kuusisto Foundation (Helsinki, Finland; grant nos. 20230026 and 20240055). The authors appreciate the staff of the Natural Resources Institute Finland (Luke) Jokioinen Research Barn, for technical support, care of experimental animals, and assistance in sample collection, and in the laboratory for chemical analysis of samples. We thank CSC-IT Center for Science (Espoo, Finland) for computational resources. Supplemental material for this article is available at http://doi.org/10.17632/tvpzgg7kgm.2. The experimental procedures were performed in accordance with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes complying with ARRIVE guidelines. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ASV = amplicon sequence variants; CT = concentrate type; EE = ether extract; FC = forage to concentrate; GE = gross energy; HF = high forage; HEE = high-EE concentrate; iNDF = indigestible NDF; LF = low forage; LEE = low-EE concentrate; NA = not analyzed; OTU = operational taxonomic units; vis = visible.

REFERENCES

Aguerre, M. J., M. A. Wattiaux, J. M. Powell, G. A. Broderick, and C. Arndt. 2011. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. J. Dairy Sci. 94:3081–3093. https://doi.org/10.3168/jds.2010-4011.

Ahvenjärvi, S., A. R. Bayat, M. Toivanen, P. Mäntysaari, and I. Tapio. 2024. The effects of residual energy intake on nutrient use, methane emissions and microbial composition in dairy cows. Sci. Rep. 14:613. https://doi.org/10.1038/s41598-024-51300-7.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

AOAC International. 2019. Official Methods of Analysis of AOAC International. 21st ed. AOAC International, Rockville, MD.

Arndt, C., A. N. Hristov, W. J. Price, S. C. McClelland, A. M. Pelaez, S. F. Cueva, J. Oh, J. Dijkstra, A. Bannink, A. R. Bayat, L. A. Crompton, M. A. Eugène, D. Enahoro, E. Kebreab, M. Kreuzer, M. McGee, C. Martin, C. J. Newbold, C. K. Reynolds, A. Schwarm, K. J. Shingfield, J. B. Veneman, D. R. Yáñez-Ruiz, and Z. Yu. 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5°C target by 2030 but not 2050. Proc. Natl. Acad. Sci. USA 119:e2111294119. https://doi.org/10.1073/pnas.2111294119.

Bayat, A. R., I. Tapio, J. Vilkki, K. J. Shingfield, and H. Leskinen. 2018. Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield. J. Dairy Sci. 101:1136–1151. https://doi.org/10.3168/jds.2017-13545.

Bayat, A. R., L. Ventto, P. Kairenius, T. Stefanski, H. Leskinen, I. Tapio, E. Negussie, J. Vilkki, and K. J. Shingfield. 2017. Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions, and nutrient utilization in lactating cows. Transl. Anim. Sci. 1:277–286. https://doi.org/10.2527/tas2017.0032.

Bayat, A. R., J. Vilkki, A. Razzaghi, H. Leskinen, H. Kettunen, R. Khurana, T. Brand, and S. Ahvenjärvi. 2022. Evaluating the effects of high-oil rapeseed cake or natural additives on methane emissions and performance of dairy cows. J. Dairy Sci. 105:1211–1224. https://doi.org/10.3168/jds.2021-20537.

Benchaar, C., G. A. Romero-Pérez, P. Y. Chouinard, F. Hassanat, M. Eugene, H. V. Petit, and C. Côrtes. 2012. Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations: Effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. J. Dairy Sci. 95:4578–4590. https://doi.org/10.3168/jds.2012-5455.

Boadi, D., C. Benchaar, J. Chiquette, and D. Massé. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84:319–335. https://doi.org/10.4141/A03-109.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson II, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857. https://doi.org/10.1038/s41587-019-0209-9.

Brask, M., P. Lund, M. R. Weisbjerg, A. L. F. Hellwing, M. Poulsen, M. K. Larsen, and T. Hvelplund. 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. J. Dairy Sci. 96:2356–2365. https://doi.org/10.3168/jds.2011-5239.

Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441–443 in Proceedings of the 3rd Symposium on Energy Metabolism of Farm Animals, European Association for Animal Production. Academic Press.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat. Methods 13:581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108(Suppl. 1):4516–4522. https://doi.org/10.1073/pnas.1000080107.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3.

Enjalbert, F., S. Combes, A. Zened, and A. Meynadier. 2017. Rumen microbiota and dietary fat: A mutual shaping. J. Appl. Microbiol. 123:782–797. https://doi.org/10.1111/jam.13501.

European Union. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. L276/33. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

Fant, P., M. Ramin, and P. Huhtanen. 2021. Replacement of barley with oats and dehulled oats: Effects on milk production, enteric methane emissions, and energy utilization in dairy cows fed a grass silage-based diet. J. Dairy Sci. 104:12540–12552. https://doi.org/10.3168/jds.2021-20409.

FAOSTAT. 2021. FAO Statistical Database. Food and Agriculture Organisation of the United Nations, Rome, Italy.

Gaffey, J., G. Rajauria, H. McMahon, R. Ravindran, C. Dominguez, M. Ambye-Jensen, M. F. Souza, E. Meers, M. M. Aragonés, D. Skunca, and J. P. M. Sanders. 2023. Green biorefinery systems for the production of climate-smart sustainable products from grasses, legumes and green crop residues. Biotechnol. Adv. 66:108168. https://doi.org/10.1016/j.biotechadv.2023.108168.

Grainger, C., and K. A. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166–167:308–320. https://doi.org/10.1016/j.anifeedsci.2011.04.021.

Haacker, K., H. J. Block, and F. Weissbach. 1983. Zur kolorimetrischen Milchsäurebestimmung in Silagen mit p-Hydroxydiphenyl. Arch. Tierernahr. 33:505–512. https://doi.org/10.1080/17450398309425704.

Hanafy, R. A., B. Johnson, N. H. Youssef, and M. S. Elshahed. 2020. Assessing anaerobic gut fungal diversity in herbivores using D1/D2 large ribosomal subunit sequencing and multi-year isolation. Environ. Microbiol. 22:3883–3908. https://doi.org/10.1111/1462-2920.15164.

Henderson, G., F. Cox, S. Ganesh, A. Jonker, and W. Young.Global Rumen Census Collaborators, and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5:14567. https://doi.org/10.1038/srep14567.

Huhtanen, P., J. Nousiainen, and M. Rinne. 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15:293–323. https://doi.org/10.2137/145960606779216317.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2008. Evaluation of concentrate factors affecting silage intake of dairy cows: A development of the relative total diet intake index. Animal 2:942–953. https://doi.org/10.1017/S1751731108001924.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92:5031–5042. https://doi.org/10.3168/jds.2008-1834.

Huhtanen, P. J., R. Blauwiekel, and I. Saastamoinen. 1998. Effects of intraruminal infusions of propionate and butyrate with two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage-based diet. J. Sci. Food Agric. 77:213–222. https://doi.org/10.1002/(SICI)1097-0010(199806)77:2<213::AID-JSFA28>3.0.CO;2-6.

Huida, L., H. Väätäinen, and M. Lampila. 1986. Comparison of dry matter contents in grass silages as determined by oven drying and gas chromatographic water analysis. Ann. Agric. Fenn. 25:215–230.

Huuki, H., S. Ahvenjärvi, P. Lidauer, M. Popova, J. Vilkki, A. Vanhatalo, and I. Tapio. 2022. Fresh rumen liquid inoculant enhances the rumen microbial community establishment in pre-weaned dairy calves. Front. Microbiol. 12:758395. https://doi.org/10.3389/fmicb.2021.758395.

IPCC. 2023. Summary for policymakers. Pages 1–34 in Climate Change 2023: AR6 Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. H. Lee and J. Romero, ed. IPCC, Geneva, Switzerland. https://doi.org/10.59327/IPCC/AR6-9789291691647.

Ishaq, S. L., and A.-D. G. Wright. 2014. Design and validation of four new primers for next-generation sequencing to target the 18S rRNA genes of gastrointestinal ciliate protozoa. Appl. Environ. Microbiol. 80:5515–5521. https://doi.org/10.1128/AEM.01644-14.

Jaakamo, M. J., T. J. Luukkonen, P. K. Kairenius, A. R. Bayat, S. A. Ahvenjärvi, T. M. Tupasela, J. H. Vilkki, K. J. Shingfield, and H. M. Leskinen. 2019. The effect of dietary forage to concentrate ratio and forage type on milk fatty acid composition and milk fat globule size of lactating cows. J. Dairy Sci. 102:8825–8838. https://doi.org/10.3168/jds.2018-15833.

Kittelmann, S., S. R. Devente, M. R. Kirk, H. Seedorf, B. A. Dehority, and P. H. Janssen. 2015. Phylogeny of intestinal ciliates, including *Charonina ventriculi*, and comparison of microscopy and 18S rRNA gene pyrosequencing for rumen ciliate community structure analysis. Appl. Environ. Microbiol. 81:2433–2444. https://doi.org/10.1128/AEM.03697-14.

Kriss, M. 1930. Quantitative relations of the dry matter of the food consumed, the heat production, the gaseous outgo, and the insensible loss in body weight of cattle. J. Agric. Res. 40:283–295.

Luke (Natural Resources Institute Finland). 2025. Finnish Feed Tables and Nutrient Requirements of Farm Animals. Natural Resources Institute Finland, Jokioinen, Finland. Accessed Apr. 1, 2025. https://www.luke.fi/en/luonnonvaratieto/science-and-information/finnish-feed-tables-and-nutrient-requirements.

Martin, C., D. P. Morgavi, and M. Doreau. 2010. Methane mitigation in ruminants: From microbe to the farm scale. Animal 4:351–365. https://doi.org/10.1017/S1751731109990620.

McCullough, H. 1967. The determination of ammonia in whole blood by direct colorimetric method. Clin. Chim. Acta 17:297–304. https://doi.org/10.1016/0009-8981(67)90133-7.

Niu, M., E. Kebreab, A. N. Hristov, J. Oh, C. Arndt, A. Bannink, A. R. Bayat, A. F. Brito, T. Boland, D. Casper, L. A. Crompton, J. Dijkstra, M. A. Eugène, P. C. Garnsworthy, M. N. Haque, A. L. F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, C. Martin, S. C. McClelland, M. McGee, P. J. Moate, S. Muetzel, C. Muñoz, P. O'Kiely, N. Peiren, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, M. R. Weisbjerg, D. R. Yáñez-Ruiz, and Z. Yu. 2018. Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Glob. Chang. Biol. 24:3368–3389. https://doi.org/10.1111/gcb.14094.

Nousiainen, J., M. Rinne, M. Hellämäki, and P. Huhtanen. 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Technol. 103:97–111. https://doi.org/10.1016/S0377-8401(02)00283-3.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, P. Solymos, M. H. H. Stevens, E. Szoecs, H. Wagner, M. Barbour, M. Bedward, B. Bolker, D. Borcard, G. Carvalho, M. Chirico, M. De Caceres, S. Durand, H. B. A. Evangelista, R. FitzJohn, M. Friendly, B. Furneaux, G. Hannigan, M. O. Hill, L. Lahti, D. McGlinn, M.-H. Ouellette, E. R. Cunha, T. Smith, A. Stier, C. J. F. T. Braak, J. Weedon, and T. Borman. 2025. Vegan: Community Ecology Package. Accessed Mar. 29, 2025. http://cran.r-project.org/package=vegan.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41:D590–D596. https://doi.org/10.1093/nar/gks1219.

Räisänen, S. E., Þ. H. Sigurðardóttir, A. Halmemies-Beauchet-Filleau, O. Pitkänen, A. Vanhatalo, A. Sairanen, and T. Kokkonen. 2024. Ruminal methane emission and lactational performance of cows fed rapeseed cake and oats on a grass silage-based diet. J. Dairy Sci. 107:6732–6741. https://doi.org/10.3168/jds.2023-24437.

Razzaghi, A., H. Leskinen, S. Ahvenjärvi, H. Aro, and A. R. Bayat. 2022. Energy utilization and milk fat responses to rapeseed oil when fed to lactating dairy cows receiving different dietary forage to concentrate ratio. Anim. Feed Sci. Technol. 293:115454. https://doi.org/10.1016/j.anifeedsci.2022.115454.

Richter, F. J., M. Suter, A. Lüscher, N. Buchmann, N. El Benni, R. Feola Conz, M. Hartmann, P. Jan, and V. H. Klaus. 2024. Effects of management practices on the ecosystem-service multifunctionality of temperate grasslands. Nat. Commun. 15:3829. https://doi.org/10.1038/s41467-024-48049-y.

Ripple, W. J., P. Smith, H. Haberl, S. A. Montzka, C. McAlpine, and D. H. Boucher. 2014. Ruminants, climate change and climate policy. Nat. Clim. Chang. 4:2–5. https://doi.org/10.1038/nclimate2081.

Saliba, L., R. Gervais, Y. Lebeuf, and P. Y. Chouinard. 2014. Effect of feeding linseed oil in diets differing in forage to concentrate ratio: 1. Production performance and milk fat content of biohydrogenation intermediates of α-linolenic acid. J. Dairy Res. 81:82–90. https://doi.org/10.1017/S0022029913000691.

Sandri, E. C., J. Lévesque, A. Marco, Y. Couture, R. Gervais, and D. E. Rico. 2020. Transient reductions in milk fat synthesis and their association with the ruminal and metabolic profile in dairy cows fed high-starch, low-fat diets. Animal 14:2523–2534. https://doi.org/10.1017/S1751731120001585.

Seedorf, H., S. Kittelmann, G. Henderson, and P. H. Janssen. 2014. RIM-DB: A taxonomic framework for community structure analysis of methanogenic archaea from the rumen and other intestinal environments. PeerJ 2:e494. https://doi.org/10.7717/peerj.494.

Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Page 156–157 in 27th Session of the International Commission for Breeding and Productivity of Milk Animals. Wageningen Academic Publishers.

Somogyi, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160:61–68. https://doi.org/10.1016/S0021-9258(18)43097-9.

Tapio, I. 2025. The effect of high-oil concentrates in high or low forage diets on rumen and fecal microbiota. Supplemental material. https://doi.org/10.17632/tvpzgg7kgm.2.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Weiss, W. P., and J. M. Pinos-Rodríguez. 2009. Production responses of dairy cows when fed supplemental fat in low- and high-forage diets. J. Dairy Sci. 92:6144–6155. https://doi.org/10.3168/jds.2009-2558.

Xu, S., L. Zhan, W. Tang, Q. Wang, Z. Dai, L. Zhou, T. Feng, M. Chen, T. Wu, E. Hu, and G. Yu. 2023. MicrobiotaProcess: A comprehensive R package for deep mining microbiome. Innovation 4:100388. https://doi.org/10.1016/j.xinn.2023.100388.

Young, D., A. Joshi, L. Huang, B. Munk, C. Wurzbacher, N. H. Youssef, M. S. Elshahed, C. D. Moon, K. Ochsenreither, G. W. Griffith, T. M. Callaghan, A. Sczyrba, M. Lebuhn, and V. Flad. 2022. Simultaneous metabarcoding and quantification of *Neocallimastigomycetes* from environmental samples: Insights into community composition and novel lineages. Microorganisms 10:1749. https://doi.org/10.3390/microorganisms10091749.

Zhang, J., H. Shi, Y. Wang, S. Li, Z. Cao, S. Ji, Y. He, and H. Zhang. 2017. Effect of dietary forage to concentrate ratios on dynamic profile changes and interactions of ruminal microbiota and metabolites in Holstein heifers. Front. Microbiol. 8:2206. https://doi.org/10.3389/fmicb.2017.02206.

ORCIDS

N. Ayanfe, https://orcid.org/0000-0002-5108-3665
I. Tapio, https://orcid.org/0000-0002-0752-9551
S. Ahvenjärvi, https://orcid.org/0000-0001-6977-0933
M. Rinne, https://orcid.org/0000-0001-6323-0661
A. Sairanen, https://orcid.org/0000-0003-1230-6288
A. R. Bayat https://orcid.org/0000-0002-4894-0662