University of Reading

Effect of Camelina sativa seeds on rumen microbiota and fermentation in dairy sheep

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

Christodoulou, C. ORCID: https://orcid.org/0000-0001-9465-3886, Mavrommatis, A., Severgnini, M., Cremonesi, P., Castiglioni, B., Kyriakaki, P., Andreaki, R., Kotsampasi, B. and Tsiplakou, E. (2026) Effect of Camelina sativa seeds on rumen microbiota and fermentation in dairy sheep. Journal of Animal Science, 104. skag033. ISSN 1525-3163 doi: 10.1093/jas/skag033 Available at https://centaur.reading.ac.uk/128568/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1093/jas/skag033

Publisher: American Society of Animal Science

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

University of Reading

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Journal of Animal Science, 2026, **104**, skag033
https://doi.org/10.1093/jas/skag033
Published: 13 February 2026
Microbiology and Microbiome

OXFORD
AMERICAN SOCIETY OF ANIMAL SCIENCE

Effect of *Camelina sativa* seeds on rumen microbiota and fermentation in dairy sheep

Christos Christodoulou[1,2], Alexandros Mavrommatis[1], Marco Severgnini[3], Paola Cremonesi[4], Bianca Castiglioni[4], Panagiota Kyriakaki[1], Rafaela Andreaki[1], Basiliki Kotsampasi[5], and Eleni Tsiplakou[1,*]

[1]Laboratory of Nutritional Physiology and Feeding, Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, Athens 11855, Greece
[2]Department of Animal Science, School of Agriculture, Policy and Development, University of Reading, Earley Gate, RG6 6EUReading, United Kingdom
[3]Institute of Biomedical Technologies—National Research Council (ITB-CNR), Segrate, 20054, Italy
[4]Institute of Agricultural Biology and Biotechnology (IBBA-CNR), Via Einstein, Lodi, 26900, Italy
[5]Research Institute of Animal Science, ELGO DIMITRA, Paralimni, Giannitsa 58100, Greece
*Corresponding author. Email: eltsiplakou@aua.gr

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Abstract

This study investigated the effect of three levels of *Camelina sativa* seeds on ewes' diet on rumen microbiota using 16S rRNA gene amplicon sequencing and biochemical assays, focusing on rumen fermentation parameters and carbohydrates, proteins, and fats metabolism. Forty-eight dairy ewes were assigned to four homogeneous groups based on the inclusion level of *C. sativa* seeds in the diet (0, 28, 51.3, and 74.6 g/kg DM; Control, CS6, CS11, and CS16, respectively). Rumen digesta were collected on the 60th day of the trial using an esophageal tube. Rumen fluid was analyzed for volatile fatty acids (VFAs) concentration and rumen enzymatic activity. In addition, rumen microbiota was characterized in both fluid and solid fractions. The acetic and propionic acid concentrations were higher ($P < 0.001$) in CS11 compared with Control and CS6. Iso-butyric, butyric, iso-valeric, valeric acid, and total VFAs concentrations were higher ($P < 0.05$) in CS11 than in Control. The acetic: propionic ratio was higher ($P = 0.003$) in Control than in CS11 and CS16. Amylase activity was lower ($P < 0.001$) in CS6. In rumen fluid, CS11 and CS16 had a lower biodiversity than the Control, while composition also differed ($P \leq 0.05$). Regarding the most notable results of the taxa relative abundance, as compared with CS11 and CS16, *Ruminobacter* and *Succinimonas* relative abundances were lower ($P < 0.05$) in CS6, while *Fibrobacter*, *Methanobrevibacter*, *Saccharofermentans*, and *Lachnospiraceae ND3007* group relative abundances were higher ($P < 0.05$). *Ruminococcus* was higher ($P < 0.001$) in CS6 than in Control. *Fibrobacter* was higher ($P < 0.05$) in the Control than in CS16. *Butyrivibrio* and *Pseudobutyrivibrio* were higher ($P < 0.001$) in CS11 compared with Control and CS6. *Selenomonas* relative abundance was higher ($P < 0.001$) in CS16 than in Control and CS6. Higher inclusion levels were associated with reduced relative abundance of fibrolytic taxa and increased abundance of microbial groups linked to carbohydrate fermentation and fatty acid biohydrogenation, with responses differing between rumen fluid and solid fractions. The lower-fat inclusion of *C. sativa* seeds could be associated with a relative enrichment of fibrolytic bacteria, potentially linked to greater phenolic bioavailability, whereas higher inclusion levels are associated with shifts toward amylolytic and propionate-associated taxa.

Lay Summary

Camelina sativa seeds have gained attention as a promising protein-energy alternative to soybean meal feed ingredient for ruminants. They also supply beneficial unsaturated fats, although their glucosinolate content may pose challenges for ruminant feeding. While many researches have focused on animal performance, few have explored the effect of *C. sativa* seeds on rumen function and microbial communities, which play a key role in digestion, nutrient use, animal performance, and greenhouse gas emissions. In this study, dairy ewes were fed diets containing different amounts of *C. sativa* seeds. Rumen content samples were collected and analyzed using 16S rRNA gene amplicon sequencing to identify changes in the microbial community. The findings show that different inclusion levels of *C. sativa* seeds in the diet can influence the balance of rumen microbes, particularly those involved in fiber degradation, carbohydrate fermentation, and the processing of dietary fats. Differences were also observed between microbes attached to feed particles and those floating in the liquid part of the rumen. While more research is needed, these findings build on earlier research and suggest that *C. sativa* seeds can be a valuable component of ruminant diets, supporting both animal productivity and sustainability goals.

Received: November 13, 2025. **Accepted:** January 29, 2026
© The Author(s) 2026. Published by Oxford University Press on behalf of the American Society of Animal Science.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Keywords *Camelina sativa*, **amplicon sequencing, rumen, microbiota**

Abbreviations: : 16S rRNA, 16S ribosomal ribonucleic acid; aNDFom, neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADF, acid detergent fiber; ADL, acid detergent lignin; CS, *Camelina sativa* seeds; Control, dietary treatment without *C. sativa* seeds; CS6, dietary treatment fed 60 g/kg of concentrate as fed *C. sativa* seeds (28.0 g/kg DM); CS11, dietary treatment fed 110 g/kg of concentrate as fed *C. sativa* seeds (51.3 g/kg DM); CS16, dietary treatment fed 160 g/kg of concentrate as fed *C. sativa* seeds (74.6 g/kg DM); DM, dry matter; DNA, deoxyribonucleic acid; fNDF, fermentable neutral detergent fiber; iNDF, indigestible neutral detergent fiber; Nel, net energy for lactation; NFC, non-fiber carbohydrates; OTU(s), operational taxonomic unit(s); zOTU(s), zero-radius operational taxonomic unit(s); PCoA, principal coordinates analysis; PUFA(s), polyunsaturated fatty acid(s); RNA, ribonucleic acid; RDP3x, rumen degradable protein estimated at 3× maintenance intake; RT-qPCR, real-time quantitative polymerase chain reaction; SEM, standard error of the mean; VFA(s), volatile fatty acid(s)

Introduction

The rumen microbiome is highly dynamic and comprises bacteria, archaea, protozoa, and fungi that work synergistically to degrade dietary components (Henderson et al. 2015; Huws et al. 2018). Modulating the rumen microbial community through nutritional management is a key research area for optimizing feed efficiency and reducing greenhouse gas emissions (Jami and Mizrahi 2012; Huws et al. 2018). *Camelina sativa* is increasingly recognized as a protein–energy feed ingredient, rather than solely a lipid source (Riaz et al. 2022). In addition to reported effects on ruminant performance, *Camelina sativa* seeds (CS) and their by-products can potentially influence rumen metabolic processes, including fatty acid biohydrogenation and nitrogen metabolism. Their relatively high crude protein content and polyunsaturated fatty acids (PUFA) profile have been associated with favorable changes, including increased PUFA concentration in milk fatty acid composition, lamb tissue fatty acid profile, and ruminal nitrogen metabolism (Hurtaud and Peyraud 2007; Cieslak et al. 2013; Brandao et al. 2018; Riaz et al. 2022). These metabolic responses are closely linked to rumen microbial activity, as specific microbial groups are involved in biohydrogenation pathways, amino acid degradation, and the production of ammonia. Hence, evaluating the effects of different forms of oilseeds (e.g., whole seeds, oils, meals) in ruminant species is essential for a comprehensive understanding of their impact on rumen fermentation and microbiota populations. Lipid-rich feeds, including oilseeds such as *C. sativa*, have attracted considerable interest due to their high concentrations of PUFA, which can alter the rumen microbiome, redirect fermentation pathways, and, in some cases, lower methane emissions (Patra 2013). More specifically, the inclusion of 60 g/kg DM of *Camelina* oil had no significant effect on bacterial population and methane yield in lactating cows (Bayat et al. 2015). Furthermore, in a dual-flow continuous culture system trial, *Camelina* seed supplementation increased propionate and reduced acetate concentrations in a dose-dependent manner (Dai et al. 2017). It also decreased the relative abundances of *Ruminococcus* spp., *Fibrobacter* spp., and *Butyrivibrio* spp., while increasing *Megasphaera* and *Succinivibrio* in the liquid fraction (Dai et al. 2017). Interestingly, the reduction in fibrolysis and populations of fibrolytic bacteria such as *Ruminococcus* spp. and *Fibrobacter* spp. can potentially be linked with PUFA toxicity (Maia et al. 2007; Dai et al. 2017). In a previous study, significant changes in targeted microbial populations were found by the quantification of specific microbial species in individual ewes' rumen fluid and solid particles fed varying levels of CS, using real-time quantitative polymerase chain reaction (RT-qPCR) (Christodoulou et al. 2023). More specifically, in the rumen solid fraction, a reduction in the relative abundance of *Ruminococcus flavefaciens*, a trend toward reduction in the relative abundance of *Fibrobacter succinogenes*, which are major fiber-degrading bacteria, as well as reductions in methanogenic archaea were found when 74.6 g/kg DM (16% inclusion level as fed) of CS were included in ewes' diets (Christodoulou et al. 2023). However, the RT-qPCR analysis limits the assessment of overall rumen microbial diversity and broader community dynamics. High-throughput sequencing provides an untargeted assessment of both abundant and low-abundance taxa, supporting the investigation of microbial groups involved in fermentation, biohydrogenation, and nitrogen metabolism (Klindworth et al. 2013; Poretsky et al. 2014).

Thus, we hypothesized that applying 16S rRNA gene amplicon next-generation sequencing would reveal broader and fraction-specific shifts in rumen microbial diversity and community composition beyond those identified by targeted RT-qPCR analysis. Therefore, the objective of this study was to apply a high-throughput sequencing approach to expand upon previous findings from the same experimental material described in Christodoulou et al. (2023), providing a deeper insight into rumen microbiota in both rumen fluid and solid fractions, as well as associated rumen fermentation characteristics in response to graded levels of PUFA-rich and protein–energy CS.

Materials and methods

Experimental design and diets

The experimental procedures used in the present study were previously described by Christodoulou et al. (2023) and were carried out in accordance with protocols approved by the Ethics Committee for Research of the Agricultural University of Athens for animal handling, housing, and care (approval no. 000007/22-01-2017). Forty-eight dairy Chios breed ewes were assigned to four homogeneous groups ($n = 12$ per treatment), balanced by age (2–4 years), body weight (55.0 ± 6.5 kg), fat-corrected (6%) milk yield (1.85 ± 0.30 kg/d), and days in milk (67 ± 8 days). Ewes were separated into different groups based on their diets and managed separately to ensure each group only experienced their assigned treatment. During feeding, within each block, ewes were moved into individual feeders to enable precise feed intake for each animal. The experimental period spanned 60 days. The diets included a daily average of 1.5 kg concentrate mix, 1.5 kg alfalfa hay, and 0.2 kg wheat straw per ewe into two meals. Fresh water was available to all ewes ad libitum. Concentrates were formulated with three levels of CS by partially replacing soybean meal and maize

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

grain. Specifically, CS were included at levels of 0% (0 g/kg DM; Control), 6% (28.0 g/kg DM; CS6), 11% (51.3 g/kg DM; CS11), and 16% (74.6 g/kg DM; CS16), respectively. The CS were incorporated as whole seeds with the rest of the concentrate ingredients into the unpelleted concentrate blends, to ensure uniform distribution at the abovementioned inclusion levels. Dietary treatment components and chemical composition are presented in Table 1. The fatty acid composition of the diets has been previously presented in Christodoulou et al. (2023).

Table 1 Dietary treatment components and chemical composition.

	Dietary treatments[1]			
	Control	**CS6**	**CS11**	**CS16**
Item[2]				
Diet components, % DM				
Forages				
Wheat straw	6.13	6.13	6.13	6.13
Alfalfa hay	47.22	47.22	47.22	47.22
Concentrates[2]				
Corn meal	16.05	13.95	12.55	11.15
Barley meal	9.33	9.33	9.33	9.33
Wheat middlings	4.67	4.67	4.67	4.67
Sunflower meal	7.46	8.40	8.40	8.40
Soybean meal	7.23	5.60	4.67	3.73
***Camelina sativa* seeds**	—	2.80	5.13	7.46
Mineral Vitamin premix	1.91	1.91	1.91	1.91
Calculated composition, % DM[3]				
Daily feed intake, g of DM	2,840	2,842	2,844	2,846
Ash	7.81	7.82	7.79	7.77
aNDFom	36.78	37.93	38.68	39.42
ADF	25.21	26.12	26.74	27.37
ADL	5.41	5.77	6.02	6.27
iNDF	12.98	13.85	14.45	15.05
fNDF	23.80	24.08	24.23	24.37
Fibrous carbohydrates	44.44	43.34	42.55	41.76
NFC	35.72	33.81	32.46	31.12
Starch	17.23	15.86	14.94	14.03
Sugars	3.41	3.4	3.38	3.36
Ether extract	1.65	2.63	3.45	4.27
Crude protein	18.12	18.19	18.23	18.27
Soluble crude protein	5.13	5.3	5.4	5.49
RDP3x	11.73	11.76	11.77	11.78
NEl, Mcal/kg	1.54	1.52	1.52	1.51
Methionine	0.29	0.3	0.3	0.31
Lysine	0.83	0.82	0.82	0.82
Lysine: Methionine	3.24:1	3.16:1	3.11:1	3.05:1
Arginine	1.02	1.06	1.1	1.13
Threonine	0.71	0.71	0.72	0.73
Leucine	1.33	1.31	1.3	1.29
Isoleucine	0.72	0.72	0.71	0.71
Valine	0.91	0.92	0.92	0.92
Histidine	0.4	0.39	0.39	0.38
Phenylalanine	0.84	0.84	0.83	0.82
Tryptophane	0.23	0.23	0.23	0.23

[1]Control = dietary treatment without *Camelina sativa* seeds; CS6 = dietary treatment fed 60 g/kg *Camelina sativa* seeds (as fed) or 28.0 g/kg DM of the concentrate; CS11 = dietary treatment fed 110 g/kg *Camelina sativa* seeds (as fed) or 51.3 g/kg DM of the concentrate; CS16 = dietary treatment fed 160 g/kg Camelina sativa seeds (as fed) or 74.6 g/kg DM of the concentrate.
[2]aNDFom = neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash; ADF = acid detergent fiber; ADL = acid detergent lignin; DM = dry matter; fNDF = fermentable neutral detergent fibre; iNDF = indigestible neutral detergent fibre; Nel = net energy for lactation; NFC = non-fiber carbohydrates; RDP3x = rumen degradable protein estimated at 3× maintenance intake.
[3]Concentrates amino acid profile (chemically analyzed, % as fed) were the following: Control, Methionine 0.27; Lysine 0.37; Glycine 1.21; Alanine 0.85; Serine 1.06; Proline 1.15; Valine 0.75; Threonine 0.78; Isoleucine 0.64; Leucine 1.42; Asparagine 1.71; Glutamine 3.77; Histidine 0.36; Phenylalanine 1.10; Arginine 1.60; Tyrosine 0.49; Cysteine 0.33. CS6, Methionine 0.34; Lysine 0.66; Glycine 1.19; Alanine 0.97; Serine 1.00; Proline 1.19; Valine 0.85; Threonine 0.76; Isoleucine 0.68; Leucine 1.46; Asparagine 2.08; Glutamine 4.39; Histidine 0.30; Phenylalanine 0.84; Arginine 1.42; Tyrosine 0.37; Cysteine 0.39. CS11, Methionine 0.33; Lysine 0.61; Glycine 1.17; Alanine 0.96; Serine 0.95; Proline 1.14; Valine 0.80; Threonine 0.72; Isoleucine 0.64; Leucine 1.34; Asparagine 2.04; Glutamine 4.34; Histidine 0.25; Phenylalanine 0.72; Arginine 1.39; Tyrosine 0.32; Cysteine 0.39. CS16, Methionine 0.35; Lysine 0.46; Glycine 1.29; Alanine 0.83; Serine 1.01; Proline 1.15; Valine 0.81; Threonine 0.77; Isoleucine 0.62; Leucine 1.29; Asparagine 1.67; Glutamine 3.87; Histidine 0.33; Phenylalanine 0.83; Arginine 1.75; Tyrosine 0.39; Cysteine 0.44.

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Sample collection

Rumen digesta samples were collected on day 60 of the experiment before feeding. Samples were collected using an electric vacuum pump at 2 mbar (MZ2CNT, Vacuubrand GmbH & Co KG, Wertheim, Germany) and a stomach tube (flexible PVC tube of 1.5 mm thickness and 10 mm I.D.) as described for sheep and goats by Ramos-Morales et al. (2014) and considering the protocols of Muizelaar et al. (2020). The stomach tube was placed at a depth up to 120 cm, while the first quantity of fluid (approx. 20–30 mL) was discarded to reduce the effect of the saliva contamination. Rumen digesta was collected in pre-warmed 1-L glass bottles and hand-shaken. Solid particles were separated from the rumen fluid using four layers of bleached cheesecloth. Immediately after collection, ensuring no saliva contamination and after quality control, 9 out of 12 samples per group were snap-frozen in liquid nitrogen and stored at −80 °C for later analysis.

Ruminal volatile fatty acid and enzymatic activity analyses

Ten mL of the rumen fluid was centrifuged at 13,000 × *g* at 4 °C for 5 min, and then the supernatant was filtered under natural pressure through a polytetrafluoroethylene 0.45 µm syringe filter (Macherey-Nagel GmbH & Co., KG, Düren, Germany) and stored in three aliquots at −80 °C until the analysis of enzymatic activities. Each aliquot was defrosted only once to ensure enzyme functionality. Alpha-amylase activity was measured using a UV/Vis spectrophotometer (GENESYS 180, Thermo Fisher Scientific, Waltham, MA, USA) as described by Mavrommatis et al. (2021). Cellulase and xylanase activities were determined using the Petri dish method according to previously described protocols (Mavrommatis et al. 2025). The ImageJ densitometry software (version 1.6, National Institute of Health, Bethesda, MD, USA) was used for clearance zone quantitative analysis. Rumen samples were also used to measure volatile fatty acid (VFA) concentrations. More specifically, 0.8 mL of rumen fluid supernatant from the previous centrifugation (without the filtration) was acidified with 0.2 mL of 25% metaphosphoric acid. After 30 min of incubation at 4 °C, samples were centrifuged at 13,000 × *g* at 4 °C for 5 min and then the supernatant was diluted with cold extra pure water (1:2). Samples were then injected in an Agilent 6890 N gas chromatograph equipped with an HP-FFAP capillary column (30 m × 0.25 mm i.d. with 0.20 µm film thickness, Agilent, Santa Clara, CA, USA) and a flame ionization detector according to previously described protocols (Mavrommatis et al. 2025).

DNA extraction and 16S amplicon sequencing

DNA was extracted from 72 samples (36 rumen fluid and 36 solid fraction samples) following the protocol by Mavrommatis et al. (2021). Briefly, 1 g of each rumen fluid or solid sample was ground into a fine powder in a mortar with liquid nitrogen. This powdered material was immediately transferred to a Falcon tube containing preheated lysis buffer and incubated at 57 °C. RNase A was then added, followed by a 37 °C incubation. DNA extraction involved three rounds of chloroform-alcohol treatment, followed by isopropanol precipitation. Overnight, samples were centrifuged at 7,500 × *g* for 15 min at 4 °C, the supernatant was discarded, and the pellet was washed twice with ethanol. The DNA pellet was then resuspended in ultrapure water and further purified using a NucleoSpin Tissue spin column (Macherey-Nagel), following the manufacturer's protocol. The extracted DNA quality was assessed based on abundance and purity (260/230 and 260/280 ratios), using an ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA), and its integrity was also verified on a 0.7% agarose gel. Following quality control assessment, six of the nine rumen samples per dietary treatment and fraction were retained for downstream analysis, resulting in a total of 48 samples (24 rumen fluid and 24 rumen solid).

Bacterial DNA was amplified according to the "16S Metagenomic Sequencing Library Preparation" protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) by Illumina (San Diego, CA, USA) using the primers described previously (Klindworth et al. 2013), which target the V3–V4 hypervariable regions of the 16S rRNA gene. All polymerase chain reaction (PCR) amplifications were performed in 25 µL volumes per sample. A total of 12.5 µL Phusion high-fidelity master mix 2 × (Thermo Fisher Scientific) and 0.2 µL of each primer (100 µM) was added to 2 µL genomic DNA as template (5 ng/µL). Blank controls (i.e., no DNA template added to the reaction) were also included. A first amplification step was performed in an Applied Biosystems 2700 thermal cycler (Thermo Fisher Scientific). The samples were denatured at 98 °C for 30 s, followed by 25 cycles with a denaturing step at 98 °C for 30 s, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. The amplicons were cleaned using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), and libraries were prepared following the 16S Metagenomic Sequencing Library Preparation Protocol (Illumina). The libraries obtained were quantified by real-time PCR using KAPA library quantification kits (Kapa Biosystems Inc., Wilmington, MA, USA), pooled in equimolar proportions, and sequenced in one MiSeq (Illumina) run with 2 × 250-bp paired-end reads. The raw 16S rRNA sequences were processed through a pipeline, including fragment rebuilding by PANDAseq (Masella et al. 2012) and quality filtering aimed at removing low-quality reads (i.e., showing stretches of bases with a Q score of <3 for more than 25% of their length). Bioinformatic analyses were conducted using the QIIME pipeline release 1.9.0 suite (Caporaso et al. 2011), clustering filtered reads into zero-radius operational taxonomic units (zOTUs) at the 97% identity level (Edgar 2016). In order to sort out putative chimeras, zOTUs supported by fewer than 5 reads across all samples were removed. Taxonomic assignment was performed by the RDP classifier (Wang et al. 2007) against the SILVA 138 database (Quast et al. 2013) using 0.8 as the confidence threshold.

Statistical analysis

The alpha diversity, which estimates the microbial species diversity on a single sample scale, was measured using the Chao1, Shannon's diversity, observed species, and Faith's phylogenetic diversity ("PD whole tree") indexes. The dataset was downsampled to the least sequenced sample to have a comparable picture of the taxonomic composition after checking the alpha-diversity rarefaction curves. A non-parametric permutation-based *t*-test (equivalent to Mann–Whitney *U*-test), with 999 random permutations, was used to assess the difference in the alpha-diversity.

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Weighted and unweighted UniFrac distances (Lozupone et al. 2011) and Principal Coordinates Analysis (PCoA) was used to represent the microbial community structure for beta-diversity, which measures the variation of microbial communities between samples (Whittaker 1960). The "Adonis" test function (Permutational Multivariate Analysis of Variance Using Distance Matrices, using pseudo-F ratios) was used to define whether there was a significant difference among the experimental groups.

For the statistical analysis of the ruminal volatile fatty acid concentration, ruminal enzymatic activity, and the ruminal relative abundances of bacterial taxa, IBM SPSS v29.0 was used based on a linear mixed model. For the ruminal volatile fatty acid concentration and ruminal enzymatic activity, the dietary treatment (Control, CS6, CS11, CS16) was the fixed factor, and the ewe's ID nested within dietary treatment was the random factor. Regarding the ruminal relative abundances of bacterial taxa, the dietary treatment (Control, CS6, CS11, CS16) and the fraction (rumen fluid and rumen solid) were the fixed factors, and the ewe's ID nested within dietary treatment was the random factor. Normality was tested using the Kolmogorov–Smirnov test, and data violating the normality assumption were log-transformed. Pairwise comparisons of estimated marginal means for fixed effects were performed, with P values adjusted using the Bonferroni correction. A Pearson correlation analysis was performed to assess relationships among VFAs, ruminal enzyme activity, and microbial genera in the rumen fluid dataset. The significance level of all tests was set at $P < 0.05$.

Results

Volatile fatty acids and enzymatic activity

The VFAs' accumulations are summarized in Table 2. Acetic and propionic acids were higher ($P < 0.001$) in CS11 than in Control and CS6. The iso-butyric, butyric, valeric acid, and total VFAs concentrations were higher ($P < 0.001$) in CS11 and CS16 compared with CS6, and in CS11 compared with Control. Iso-valeric acid was higher ($P = 0.017$) in CS11 than in Control. The acetic: propionic ratio was higher ($P = 0.003$) in Control than in CS11 and CS16.

The ruminal enzymatic activity is presented in Table 2. Amylase activity was lower ($P < 0.001$) in CS6 compared to the other dietary groups. Cellulase and xylanase activities did not differ ($P > 0.05$).

Rumen microbial communities

The amplicon-based 16S rRNA next-generation sequencing allowed us to characterize the main constituents of the rumen microbiota (Figures S1 and S2; see online supplementary material for a color version of these figure).

At the phylum level, *Bacteroidota* was the most abundant taxa, both for the fluid and the solid fractions (average rel. ab: 43.5% vs. 39.3%, respectively), followed by *Firmicutes* (27.6% vs.31.0%, respectively), *Proteobacteria* (11.5% vs. 6.0%, respectively), *Spirochaetota* (1.8% vs. 5.6%), *Verrucomicrobiota* (5.7% vs. 3.3%), and *Fibrobacterota* (2.4% vs. 5.3%, respectively).

At family level, the samples all showed a similar composition, with *Prevotellaceae* (29.3% vs. 30.0%), *Succinivibrionaceae* (10.7% vs. 5.2%), *Lachnospiraceae* (7.1% vs. 9.1%), and *Rikenellaceae* (5.3% vs. 5.6%) as the main groups of the rumen microbiota; uncultured rumen bacteria from WCHB1-41 (*Kirimatellae*), *Bacteroidales* RF16 group, *Acidaminococcacea*, *Spirochaetaceae*, *Fibrobacteraceae*, *Oscillospiraceae*, and *Ruminococcaceae* were also consistently present.

At genus level, among the main genera, we observed *Prevotella*, *Succiniclasticum*, *Fibrobacter*, and *Treponema*. In contrast, many other taxa remained unresolved at genus level (e.g., *Succinivibrionaceae* UCG-002, *Rikenellaceae* RC9 gut group, uncultured rumen bacteria from WCHB1-41, *Bacteroidales* RF16 group, *Prevotellaceae* UCG-001), due to the relatively worse characterization of the rumen in the reference databases.

The analysis of the rarefaction curves for the Chao1 metric determined that all the samples (both fluid and solid fractions) showed a

Table 2 Ruminal volatile fatty acid accumulation and enzymatic activities in ewes fed four dietary treatments with different levels of *Camelina sativa* seeds.

	Dietary treatments[1]					
	Control *n* = 6	CS6 *n* = 6	CS11 *n* = 6	CS16 *n* = 6	SEM	*P* value[2]
Volatile fatty acids, mM						
Acetic	35.6[b]	21.3[c]	49.5[a]	42.1[ab]	2.99	<0.001
Propionic	8.18[bc]	4.87[b]	13.2[a]	11.6[ab]	0.885	<0.001
Butyric	7.90[b]	3.59[c]	11.4[a]	9.57[ab]	0.874	<0.001
Iso-butyric	1.08[bc]	1.00[c]	1.80[a]	1.47[ab]	0.112	<0.001
Valeric	0.51[bc]	0.34[c]	0.81[a]	0.70[ab]	0.065	<0.001
Iso-valeric	0.96[b]	1.14[ab]	2.58[a]	1.14[ab]	0.613	0.017
Total VFAs	54.2[b]	32.2[c]	79.2[a]	66.5[ab]	4.697	<0.001
Acetic: Propionic	4.55[a]	4.39[ab]	3.81[bc]	3.67[c]	0.175	0.003
Enzymatic activities						
Cellulase, units/mL/h	21.2	20.4	21.0	20.5	0.29	0.291
Amylase, mg maltose release/mL	1.86[a]	1.15[b]	2.73[a]	1.87[a]	0.258	<0.001
Xylanase, units/mL/h	16.6	15.6	16.0	16.2	0.28	0.160

[1]Control = dietary treatment without Camelina sativa seeds; CS6 = dietary treatment fed 60 g/kg Camelina sativa seeds (as fed) or 28.0 g/kg DM of the concentrate; CS11 = dietary treatment fed 110 g/kg Camelina sativa seeds (as fed) or 51.3 g/kg DM of the concentrate; CS16 = dietary treatment fed 160 g/kg Camelina sativa seeds (as fed) or 74.6 g/kg DM of the concentrate.
[2]Significances were declared at $P < 0.05$. Significant differences between dietary treatments within variables are indicated with different superscript letters according to the Bonferroni test.

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

tendency toward reaching a plateau at around 5,000–10,000 reads, suggesting that this number of sequences was sufficient to capture the majority of the ecosystem composition. Samples' biodiversity (alpha-diversity) had a trend toward a reduction with increasing CS inclusion levels, with the CS11 and CS16 having a lower diversity than the Control (chao1, Shannon, PD whole tree, observed species metrics; $P \leq 0.036$ for all metrics). Furthermore, CS11 and CS16 composition (unweighted UniFrac distance) differed ($P=0.039$) compared with the Control and CS6 (Figure 1, panels A–B).

Rumen solid sample biodiversity (alpha diversity) did not differ among the dietary treatments. Regarding the microbial composition, this was different for both unweighted and weighted UniFrac distances for Control ($P \leq 0.007$) and CS6 ($P \leq 0.004$), and these two groups were separated from CS11 and CS16 (Figure 1, panels C–D).

The results for the relative abundances at the phylum level are presented in Table 3. More specifically, Proteobacteria relative abundance was higher ($P<0.001$) in CS16 compared with the Control and CS6, and in CS11 than in CS6. *Verrucomicrobiota*, *Fibrobacterota*, and *Euryachaeota* relative abundances were higher ($P<0.001$) in CS6 compared with CS11 and CS16. *Verrucomicrobiota* relative abundance was also higher ($P<0.001$) in Control than in CS11 and CS16. Furthermore, the fraction effect was significant ($P<0.05$) for every phylum except for *Patescibacteria*. More specifically, *Bacteroidota* and *Proteobacteria* were more abundant in the fluid than in the solid, while the remaining phyla showed the

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

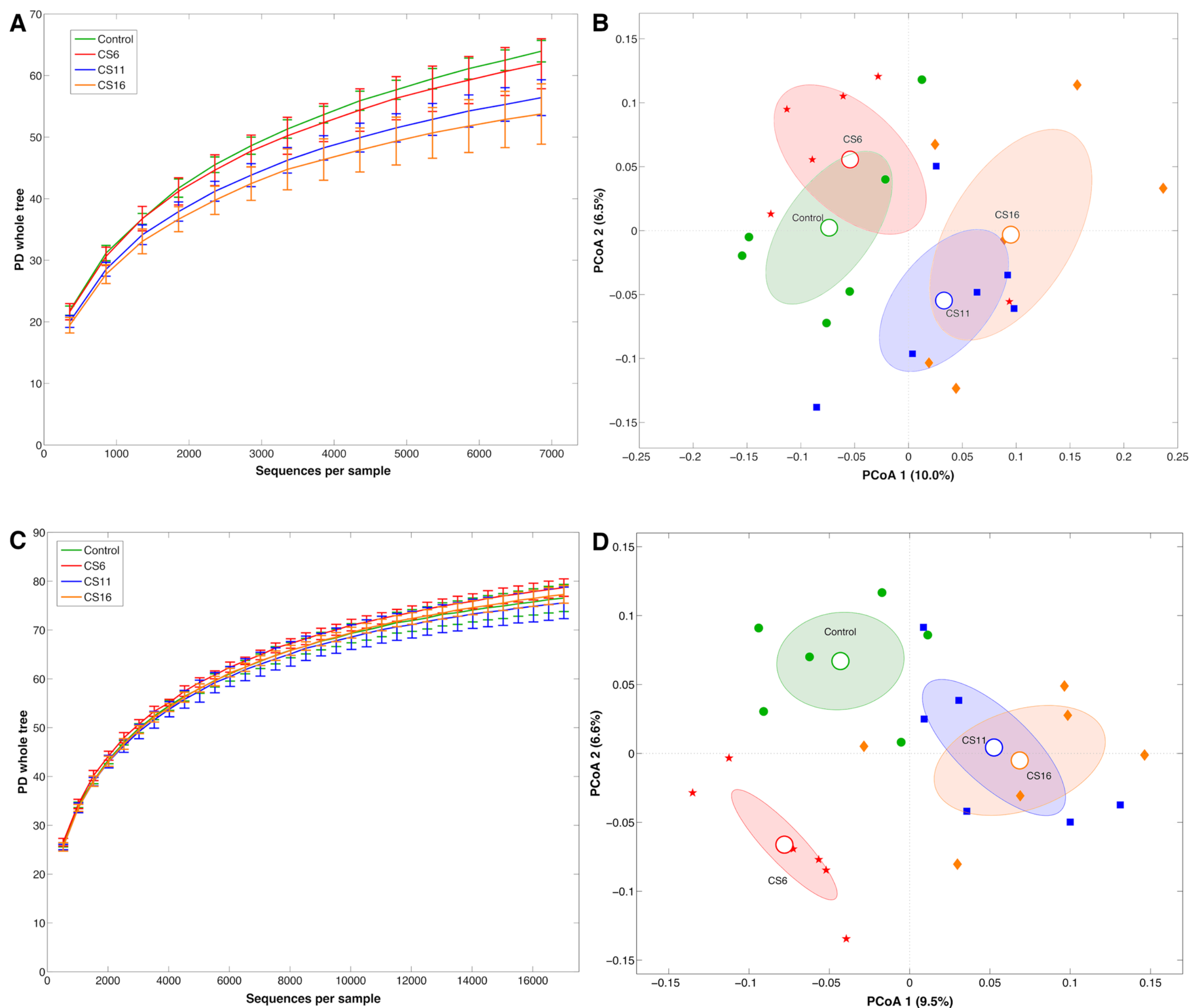

Figure 1 A) Rarefaction curves of the alpha-diversity of the samples, estimated by Faith's phylogenetic diversity metric, for the fluid part of the rumen microbiota; each line is the average value over all the samples within the same experimental group (i.e., diet); error bars represent standard errors. B) Principal Coordinate Analysis (PCoA) plot based on the unweighted UniFrac distance among samples for the fluid part of the rumen microbiota; each point represents a sample, colored according to the experimental group (i.e., diet); ellipses are the SEM-based confidence intervals, and centroids are the average coordinate of all the samples in the same group. C) Rarefaction curves of the alpha-diversity of the samples, estimated by Faith's phylogenetic diversity metric, for the solid part of the rumen microbiota; each line is the average value over all the samples within the same experimental group (i.e., diet); error bars represent standard errors. D) Principal Coordinate Analysis (PCoA) plot based on the unweighted UniFrac distance among samples for the solid part of the rumen microbiota; each point represents a sample, colored according to the experimental group (i.e., diet); ellipses are the SEM-based confidence intervals, and centroids are the average coordinates of all the samples in the same group.

opposite behaviour (Table 3). The dietary treatment × fraction interaction was not significant (Table 3).

At the family level, we detected a significant ($P < 0.05$) increase of *Prevotellaceae* and *Succinivibrionaceae* in both CS11 and CS16, of *Lachnospiraceae* in CS11 only, and of *Selenomonadaceae* in CS16 only. At the same time, *Rikenellaceae*, *Bacteroidales* RF16 group, *Acidaminococcaceae*, F082, and *Methanobacteriaceae* were higher in CS6, as well as Uncultured rumen bacterium (WCHB1-41) *Fibrobacteraceae*, *Christensenellaceae*, and *Hungateiclostridaceae* were higher in Control and CS6 compared with samples from ewes fed a higher camelina concentration. Regarding the fraction effect, it was found to be significant for most of the families (Table 3). The dietary treatment × fraction interaction was significant ($P = 0.001$) for *Christensenellaceae*.

Significant ($P < 0.05$) effects of treatment, fraction, and their interaction at the genus level are presented in Table 3. In detail, *Prevotella* and *Lachnospiraceae (other)* were higher ($P < 0.001$) in CS11 and CS16 than in Control and CS6. Furthermore, *Ruminococcaceae (other)*, *Succinivibrionaceae UCG-002*, *Ruminobacter*, and *Succinimonas* relative abundances were lower in CS6. *Rikenellaceae RC9 gut group* and *uncultured rumen bacterium (F082)* relative abundances were higher in CS6. *Uncultured rumen bacterium (WCHB1-41)* and *Christensenellaceae R-7 group* were higher ($P < 0.001$) in Control and CS6. *Lachnospiraceae AC2044 group* was higher in CS6 than in CS16. *Fibrobacter*, *Methanobrevibacter*, *Saccharofermentans*, and *Lachnospiraceae ND3007* group relative abundances were higher in CS6 than in CS11 and CS16. *Fibrobacter* and *Saccharofermentans* were also higher in the Control compared with the CS16. *Succiniclasticum* and *Methanosphaera* were higher in CS6 than in CS11. *Ruminococcus* was higher ($P < 0.001$) in CS6 than in Control. *Succinivibrionaceae UCG-001*, *Anaerovibrio*, and *Prevotellaceae YAB2003 group* were higher in CS16 compared with CS6. *Butyrivibrio* and *Pseudobutyrivibrio* were higher ($P < 0.001$) in CS11 compared with Control and CS6. *Selenomonas* and *Lachnospiraceae NK3A20 group* relative abundances were higher in CS16 than in Control and CS6. The fraction effect was found significant for most of the genus (Table 3), while the dietary treatment × fraction interaction was found significant ($P < 0.05$) for *Christensenellaceae R-7 group*, *Butyrivibrio*, *Selenomonas*, *Lachnospiraceae XPB1014 group*, *Prevotellaceae Ga6A1 group*, and *Methanosphaera* (Table 3).

Correlation analysis

Core genera, including *Prevotella*, *Succinivibrionaceae UCG-002*, *Selenomonas*, *Ruminococcaceae (other)*, and *Succinimonas* were positively correlated with acetic and propionic acids ($P < 0.05$). In contrast, *Rikenellaceae RC9 gut group*, *Fibrobacter*, *Methanobrevibacter*, *F082 (other)*, *Lachnospiraceae ND3007 group*, *Desulfovibrio*, and *Lachnospiraceae NK4A136 group* were negatively correlated with acetic, propionic, butyric, and total VFAs and other VFAs. In addition, amylase was positively correlated with *Succinivibrionaceae UCG-002*, *Ruminobacter, Ruminococcaceae (other)*, *Pserudobutyrivibrio*, *Lachnospiraceae AC2044 group*, and *Lachnospiraceae XPB1014 group*, while it was negatively correlated with *F082 (other)*. Cellulase was negatively correlated with *Treponema* (File S2).

Discussion

Studying rumen microbial structure is crucial for understanding the complex interactions between diet, microbial populations, and host physiology, which directly influence fermentation efficiency, nutrient utilization, and overall animal homeostasis. This study, which shares the same experimental conditions as Christodoulou et al. (2023), builds upon those findings, which were based on RT-qPCR analysis of selected microbial taxa, by applying an untargeted and more detailed high-throughput sequencing approach. By employing 16S rRNA gene amplicon sequencing, this study provides a more comprehensive understanding of microbial dynamics, capturing the broader diversity and interactions within the rumen microbiota in both the fluid and solid fractions, as well as the distinct microbial profiles of each fraction. The alpha-diversity analysis indicated a shift toward less diverse microbial communities in the rumen fluid with increasing levels of CS, particularly in CS11 and CS16. The distinct beta-diversity observed between these higher levels of inclusion and the lower ones suggests significant compositional changes driven by diet. Regarding the rumen solid fraction, the stability in alpha-diversity contrasted with the compositional shifts between the dietary treatments. The different clustering of the Control and CS6 from CS11 and CS16 highlighted the diet-specific impact on the solid-associated microbiota.

As expected, the dominant phyla were *Bacteroidota*, *Firmicutes*, and *Proteobacteria*, a finding consistent with the literature (Morgavi et al. 2015). In parallel, the reduction in *Fibrobacterota* and *Verrucomicrobiota* relative abundances found in the higher *C. sativa* seed inclusion levels highlight a potentially crucial trade-off between fiber degradation and carbohydrate utilization, especially since microbes belonging to *Fibrobacterota* are critical for fiber degradation.

Considering the family level, *Prevotellaceae*, which increased at higher *C. sativa* levels, is one of the most dominant families in the rumen microbiome, including bacteria of the genus *Prevotella*, which play a significant role in carbohydrate and protein degradation (Griswold et al. 1999). Microbes within this family are involved in the degradation of starch, simple sugars, and protein, producing VFAs and organic acids such as acetate and succinate, respectively, as their principal fermentation products, with lesser production of branched-chain VFAs like iso-butyric and iso-valeric, as well as lactic acid (Tett et al. 2021; Cheng et al. 2022). Therefore, their increase in CS16 could potentially be linked with facilitated carbohydrate and protein degradation. Although the decline in *Fibrobacteraceae* (key fibrolytic taxa), including the genus *Fibrobacter*, which confirms previous data (Dai et al. 2017) may suggest reduced fibrolytic potential, the increase in *Lachnospiraceae*, some of which exhibit fibrolytic traits (Palevich et al. 2019) may represent a shift in fiber degradation rather than an overall suppression of fibrolysis. Microbes of the *Lachnospiraceae* family produce hydrogen during fermentation (Kaminsky et al. 2023), creating a natural competition for hydrogen between methanogens and other hydrogen-consuming microorganisms in the rumen. In addition, the increased abundance of *Succinivibrionaceae* in CS16 suggests enhanced succinate fermentation (Russell and Rychlik 2001), likely driven by increased availability of fermentable substrates, as members of this family

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Table 3 Relative abundance (%) of bacteria taxa in ewes' rumen of the four dietary treatments fed different levels of *Camelina sativa* seeds.

% Relative abundance	Dietary treatments, *D*[1]					Fraction, *F*			*P* value[2]		
	Control $n=12$	CS6 $n=12$	CS11 $n=12$	CS16 $n=12$	SEM	Fluid $n=24$	Solid $n=24$	SEM	*D*	*F*	*D*×*F*
Phylum											
Bacteroidota	41.2	41.4	41.5	41.5	0.56	43.5	39.3	0.36	0.978	<0.001	0.214
Firmicutes	28.3	30.0	29.8	29.2	0.76	27.6	31.1	0.47	0.422	<0.001	0.175
Proteobacteria	8.16[b]	4.04[c]	11.2[ab]	12.1[a]	0.761	11.8	5.99	0.535	<0.001	<0.001	0.229
Verrucomicrobiota	4.41[a]	4.93[a]	2.87[b]	3.13[b]	0.240	2.35	5.32	0.163	<0.001	<0.001	0.455
Fibrobacterota	4.41[a]	4.93[a]	2.87[b]	3.13[b]	0.240	2.35	5.32	0.163	<0.001	<0.001	0.065
Patescibacteria	2.05	2.46	2.10	1.81	0.179	2.09	2.12	0.115	0.120	0.833	0.543
Spirochaetota	3.93	3.38	3.90	3.65	0.221	1.85	5.58	0.156	0.281	<0.001	0.236
Euryachaeota	2.17[ab]	2.75[a]	1.54[b]	1.47[b]	0.197	1.45	2.52	0.119	<0.001	<0.001	0.777
Chloroflexi	0.96	1.09	0.90	0.69	0.100	0.79	1.03	0.071	0.055	0.019	0.997
Family											
Prevotellaceae	26.6[bc]	25.2[c]	28.0[ab]	28.8[a]	0.48	29.3	25.0	0.34	<0.001	<0.001	0.894
Succinivibrionaceae	7.13[b]	2.85[c]	10.3[ab]	11.3[a]	0.833	10.7	5.15	0.579	<0.001	<0.001	0.162
Lachnospiraceae	6.96[c]	7.61[bc]	9.47[a]	8.45[ab]	0.343	7.12	9.12	0.232	<0.001	<0.001	0.069
Rikenellaceae	5.67[ab]	5.94[a]	5.29[ab]	5.04[b]	0.188	5.35	5.62	0.127	0.015	0.126	0.683
Uncultured rumen bacterium (WCHB1-41)	4.60[a]	5.30[a]	2.92[b]	3.11[b]	0.377	5.17	2.79	0.256	<0.001	<0.001	0.466
Bacteroidales RF16 group	2.92[ab]	3.69[a]	2.42[b]	2.59[ab]	0.271	4.09	1.72	0.177	0.016	<0.001	0.056
Ruminococcaceae	3.83	3.52	4.09	4.13	0.190	3.83	3.95	0.130	0.119	0.489	0.906
Acidaminococcaceae	3.35[ab]	3.83[a]	2.88[b]	3.22[ab]	0.211	3.44	3.20	0.148	0.033	0.253	0.300
Oscillospiraceae	3.76	4.01	3.63	3.13	0.252	3.09	4.18	0.156	0.126	<0.001	0.064
F082	3.35[ab]	4.11[a]	3.19[b]	2.84[b]	0.228	2.98	3.77	0.162	0.003	0.001	0.886
Selenomonadaceae	1.22[b]	1.17[b]	1.59[ab]	2.17[a]	0.207	2.52	0.55	0.141	0.010	<0.001	0.258
Fibrobacteraceae	4.41[a]	4.93[a]	2.87[b]	3.13[b]	0.240	2.35	5.32	0.163	<0.001	<0.001	0.065
Spirochaetaceae	3.92	3.37	3.89	3.64	0.220	1.84	5.57	0.156	0.274	<0.001	0.232
Methanobacteriaceae	2.17[ab]	2.75[a]	1.54[b]	1.47[b]	0.197	1.45	2.52	0.119	<0.001	<0.001	0.777
Uncultured rumen bacterium (Absconditabacteriales [SR1])	0.85	1.28	1.24	1.09	0.120	1.13	1.10	0.075	0.066	0.726	0.164
Clostridia UCG-014 (other)	0.90	0.89	1.20	1.37	0.130	1.11	1.07	0.086	0.040	0.763	0.792
Christensenellaceae	1.56[ab]	1.66[a]	1.28[bc]	1.23[c]	0.071	0.97	1.89	0.050	<0.001	<0.001	0.001
Muribaculaceae	1.17	0.82	1.21	1.01	0.167	0.70	1.40	0.107	0.357	<0.001	0.977
Hungateiclostridaceae	1.00[ab]	1.09[a]	0.83[bc]	0.75[c]	0.057	0.70	1.13	0.039	0.002	<0.001	0.224
Anaerolineaceae	0.96	1.09	0.90	0.69	0.100	0.79	1.03	0.071	0.055	0.019	0.997

(Continued)

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Table 3 Continued.

% Relative abundance	Dietary treatments, D[1]				SEM	Fraction, F		SEM	P value[2]		
	Control $n=12$	CS6 $n=12$	CS11 $n=12$	CS16 $n=12$		Fluid $n=24$	Solid $n=24$		D	F	$D \times F$
Genus											
Prevotella	18.4^{b}	17.1^{b}	20.1^{a}	20.4^{a}	0.41	21.5	16.6	0.29	<0.001	<0.001	0.693
Succinivibrionaceae UCG-002	4.27^{a}	1.46^{b}	5.70^{a}	5.97^{a}	0.528	5.69	3.01	0.361	<0.001	<0.001	0.222
Rikenellaceae RC9 gut group	5.59^{b}	5.86^{a}	5.23^{b}	4.98^{b}	0.188	5.30	5.53	0.126	0.017	0.188	0.716
Uncultured rumen bacterium (WCHB1-41)	4.60^{a}	5.30^{a}	2.92^{b}	3.11^{b}	0.377	5.17	2.79	0.256	<0.001	<0.001	0.303
Fibrobacter	4.41^{ab}	4.93^{a}	2.87^{bc}	3.13^{c}	0.240	2.35	5.32	0.163	<0.001	<0.001	0.065
Succiniclasticum	3.34^{ab}	3.83^{a}	2.87^{b}	3.21^{ab}	0.211	3.44	3.20	0.148	0.011	0.254	0.247
Prevotellaceae UCG-001	2.57	2.36	2.57	2.75	0.213	2.41	2.72	0.151	0.643	0.147	0.683
Treponema	3.73	3.13	3.80	3.53	0.218	1.70	5.39	0.154	0.144	<0.001	0.145
NK4A214 group	2.27	2.68	2.44	2.13	0.143	1.99	2.77	0.098	0.064	<0.001	0.052
Succinivibrionaceae UCG-001	1.84^{ab}	0.35^{b}	2.25^{ab}	3.80^{a}	0.649	1.08	3.05	0.459	0.006	0.004	0.384
Bacteroidales RF16 group	1.74	2.25	1.42	1.58	0.205	2.52	0.97	0.132	0.052	<0.001	0.081
Methanobrevibacter	2.05^{ab}	2.61^{a}	1.46^{b}	1.38^{b}	0.162	1.42	2.33	0.115	<0.001	<0.001	0.898
Prevotellaceae (other)	1.77	1.72	1.81	1.90	0.118	2.27	1.33	0.078	0.753	<0.001	0.319
Ruminococcus	1.49^{b}	1.95^{a}	1.62^{ab}	1.79^{ab}	0.132	1.25	2.17	0.043	<0.001	0.480	0.480
Prevotellaceae UCG-003	1.50	1.74	1.46	1.49	0.088	1.42	1.68	0.062	0.107	0.005	0.429
Ruminobacter	1.45^{a}	0.47^{b}	2.25^{a}	1.54^{a}	0.306	1.66	1.19	0.207	<0.001	0.168	0.427
Ruminococcaceae (other)	1.54^{a}	0.86^{b}	1.60^{a}	1.45^{a}	0.134	1.57	1.16	0.089	<0.001	0.004	0.204
Christensenellaceae R-7 group	1.49^{a}	1.60^{a}	1.17^{b}	0.46^{c}	0.063	0.94	1.42	0.045	<0.001	<0.001	<0.001
F082 (other)	1.37	1.44	0.99	0.07	0.112	0.00	1.39	0.079	0.006	<0.001	0.659
Lachnospiraceae (other)	1.05^{b}	0.98^{b}	1.79^{a}	1.59^{a}	0.137	1.39	1.31	0.097	<0.001	0.878	0.696
Butyrivibrio	1.01^{b}	0.95^{b}	1.45^{a}	1.19^{ab}	0.076	1.02	1.27	0.053	<0.001	0.002	0.011
Clostridia UCG-014 (other)	0.90	0.89	1.20	1.37	0.121	1.11	1.08	0.086	0.018	0.795	0.841
Uncultured rumen bacterium (Absconditabacteriales [SR1])	0.85	1.28	1.24	1.09	0.106	1.13	1.10	0.075	0.028	0.775	0.273
Uncultured rumen bacterium (F082)	0.91^{b}	1.46^{a}	0.98^{b}	0.87^{b}	0.086	1.03	1.08	0.061	<0.001	0.640	0.598
Flexilinea	0.96	1.09	0.90	0.69	0.100	0.79	1.03	0.071	0.055	0.019	0.997
Saccharofermentans	0.86^{ab}	0.98^{a}	0.72^{bc}	0.64^{c}	0.052	0.60	1.01	0.036	<0.001	<0.001	0.182
Uncultured rumen bacterium (Muribaculaceae)	0.86	0.70	0.95	0.72	0.136	0.53	1.09	0.083	0.522	<0.001	0.867
Prevotellaceae UCG-004	0.86	0.82	0.69	0.84	0.073	0.60	1.01	0.046	0.351	<0.001	0.202
[Eubacterium] ruminantium group	0.70	0.79	0.89	0.78	0.071	0.67	0.91	0.045	0.296	<0.001	0.052

(Continued)

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Table 3 Continued.

% Relative abundance	Dietary treatments, D[1]				SEM	Fraction, F		SEM	P value[2]		
	Control $n=12$	CS6 $n=12$	CS11 $n=12$	CS16 $n=12$		Fluid $n=24$	Solid $n=24$		D	F	$D \times F$
Selenomonas	0.41^{b}	0.36^{b}	0.76^{ab}	1.14^{a}	1.135	1.13	0.20	0.096	<0.001	<0.001	0.011
Succinivibrio	0.56	0.54	0.76	0.91	0.105	1.09	0.29	0.071	0.064	<0.001	0.884
Lachnospiraceae AC2044 group	0.42^{ab}	0.57^{a}	0.52^{ab}	0.38^{b}	0.050	0.30	0.64	0.040	0.036	<0.001	0.550
Lachnospiraceae NK3A20 group	0.43^{b}	0.43^{b}	0.46^{ab}	0.57^{a}	0.032	0.37	0.57	0.022	0.020	<0.001	0.077
Pseudobutyrivibrio	0.35^{b}	0.30^{b}	0.68^{a}	0.51^{ab}	0.053	0.43	0.48	0.037	<0.001	0.351	0.215
Selenomonadaceae (other)	0.27	0.25	0.25	0.31	0.043	0.469	0.074	0.028	0.725	<0.001	0.657
Prevotellaceae NK3B31 group	0.26	0.26	0.22	0.21	0.032	0.14	0.34	0.022	0.557	<0.001	0.598
Lachnospiraceae ND3007 group	0.23^{ab}	0.38^{a}	0.19^{b}	0.19^{b}	0.043	0.31	0.19	0.028	0.015	0.002	0.602
Lachnospiraceae XPB1014 group	0.21	0.23	0.26	0.20	0.026	0.22	0.23	0.017	0.316	0.811	0.043
Succinimonas	0.19^{a}	0.08^{b}	0.20^{a}	0.23^{a}	0.036	0.22	0.13	0.025	0.002	0.046	0.572
Anaerovibrio	0.19^{ab}	0.18^{b}	0.19^{ab}	0.27^{a}	0.033	0.35	0.07	0.023	0.095	<0.001	0.188
Prevotellaceae YAB2003 group	0.15^{ab}	0.11^{b}	0.12^{ab}	0.26^{a}	0.039	0.19	0.13	0.027	0.019	0.039	0.100
Lachnospiraceae UCG-008	0.19	0.26	0.21	0.19	0.024	0.18	0.25	0.017	0.153	0.010	0.443
Desulfovibrio	0.12	0.17	0.12	0.09	0.022	0.12	0.13	0.015	0.121	0.469	0.874
Lachnospiraceae NK4A136	0.12	0.16	0.12	0.15	0.016	0.09	0.19	0.012	0.137	<0.001	0.078
Blautia	0.14	0.17	0.14	0.13	0.017	0.06	0.23	0.012	0.303	<0.001	0.379
Methanosphaera	0.11^{ab}	0.15^{a}	0.08^{b}	0.09^{ab}	0.016	0.03	0.18	0.010	0.040	<0.001	0.019
Prevotellaceae Ga6A1 group	0.09	0.05	0.14	0.07	0.023	0.05	0.12	0.015	0.084	<0.001	0.008
Moraxella	0.07	0.10	0.06	0.08	0.023	0.06	0.09	0.016	0.491	0.248	0.800
Lachnospiraceae UCG-006	0.06	0.05	0.04	0.03	0.009	0.04	0.05	0.007	0.585	0.688	0.250
Lachnospiraceae FCS020 group	0.05	0.08	0.07	0.05	0.010	0.04	0.08	0.007	0.251	<0.001	0.283

[1]Control = dietary treatment without Camelina sativa seeds; CS6 = dietary treatment fed 60 g/kg Camelina sativa seeds (as fed) or 28.0 g/kg DM of the concentrate; CS11 = dietary treatment fed 110 g/kg Camelina sativa seeds (as fed) or 51.3 g/kg DM of the concentrate; CS16 = dietary treatment fed 160 g/kg Camelina sativa seeds (as fed) or 74.6 g/kg DM of the concentrate.
[2]Significances were declared at $P < 0.05$. Significant differences between dietary treatments within variables are indicated with different superscript letters according to the Bonferroni test.

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

produce succinate as the principal fermentation end product (Lee et al. 1999).

Several notable changes in microbial abundance were observed at the genus level, reflecting the influence of *C. sativa* seed inclusion levels on the rumen microbiota in both fluid and solid compartments. Among the main genera observed in both rumen fluid and solid particles were *Prevotella*, *Succiniclasticum*, and *Fibrobacter*, with *Treponema* additionaly present in the solid fraction. However, many other taxa remained unresolved at the genus level in both compartments (e.g., *Succinivibrionaceae UCG-002*, *Rikenellaceae RC9 gut group*, *uncultured rumen bacteria from WCHB1-41*, *Bacteroidales RF16 group*, *Oscillospiraceae NK4A214 group*, *Prevotellaceae UCG-001*), due to the relatively more limited characterization of the rumen microbiota in the reference databases. The increased abundance of *Prevotella* in the highest inclusion level (CS16) could indicate enhanced fermentation of readily fermentable substrates, which may contribute to altered fermentation patterns (Betancur-Murillo et al. 2022). While *Prevotella* relative abundance was not significantly altered in the targeted RT-qPCR analysis of Christodoulou et al. (2023), the 16S amplicon sequencing approach of this study revealed increased *Prevotella* relative abundance with higher CS inclusion. Considering the correlation analysis, the positive correlations between *Prevotella* and propionic acids may suggest an active role of *Prevotella* in carbohydrate fermentation and VFA production. Similar associations have been reported previously, particularly for *Prevotella* and *Selenomonas*, which are known to contribute to propionate formation and efficient energy use in the rumen. This increase may also reflect an inhibitory fibrolytic activity, in line with the reduced *Fibrobacter* abundance, suggesting a potential decrease in fiber degradation efficiency at higher inclusion levels of CS. This reduction may be attributed to the altered nutrient profile associated with increased *Camelina* inclusion, which could selectively favor or inhibit specific microbial groups. For instance, dietary PUFA can influence ruminal fatty acid biohydrogenation by altering the abundance of microbial groups involved in lipid metabolism. It is essential to link the results of this study and interpret the ruminal lipid metabolism with the ruminal fatty acid profiles reported by Christodoulou et al. (2023), which were generated from the same experimental animals and dietary treatments. Interestingly, increasing the inclusion of CS increased the ruminal availability of unsaturated fatty acids, particularly cis-9 $C_{18:1}$. While biohydrogenation pathways remained active, as indicated by the presence of trans-$C_{18:1}$ intermediates and responsive biohydrogenation-associated taxa at moderate inclusion levels, the fatty acid profile may suggest that the capacity for complete biohydrogenation was exceeded under higher PUFA supply. Specifically, cis-9 $C_{18:1}$ was likely subjected to biohydrogenation but not fully converted to $C_{18:0}$, consistent with a limitation at the terminal reduction step. The absence of a linear increase in *Butyrivibrio* abundance at the highest CS level further supports the interpretation of functional saturation rather than enhanced biohydrogenation capacity. Furthermore, PUFA toxicity has been linked to reduced cellulolytic activity (Maia et al. 2007; Dai et al. 2017). The reduction in *Fibrobacter* at higher *C. sativa* inclusion levels is consistent with the RT-qPCR results of Christodoulou et al. (2023), supporting the sensitivity of fiber-associated microbial populations to PUFA-rich oilseed supplementation. The potential PUFA-toxicity toward cellulolytic taxa such as *Fibrobacter* has been reported in goats fed oilseeds such as linseed (Abuelfatah et al. 2016) or dairy cows fed whole or ground flaxseed (Huang et al. 2021). Despite the observed reduction in such a key fibrolytic microbe, in this study, cellulase enzymatic activity remained unaffected, suggesting that fiber degradation may have been preserved, or that the sample collection time was not indicative of cellulase and xylanase activity, which typically occurs several hours after feeding. Plant cell wall polysaccharides are degraded during the later stages of ruminal fermentation, after soluble carbohydrates have been utilized and fibrolytic microbes have grown (Martin and Michalet-Doreau 1995). Interestingly, unclassified members of the *Ruminococcaceae* had a higher abundance in CS11 and CS16 compared to CS6, contrasting with the previously discussed decline in fiber-degrading microbes, particularly *Fibrobacteraceae*. This suggests the competition within the rumen microbiota (niche expansion theory), which could help balance fermentation pathways and the rate of carbohydrate degradation (Nagaraja 2016; Owens and Basalan 2016). Together, these results suggest that the extent of PUFA release in the rumen, rather than the specific oilseed source, plays a central role in shaping rumen microbial responses. In this study, the reduction in *Ruminobacter*, with potent amylolytic activity, alongside an increase in fiber-degrading groups like *Ruminococcus* and *Fibrobacter*, might be linked to the combined effects of plant compounds from CS and the lower ether extract content in the CS6 diet compared with CS11 and CS16. Moreover, CS contain phenolic compounds and glucosinolates. These compounds can bind to microbial enzymes and interact with nutrients such as starch and protein, exerting antimicrobial effects (Giuberti et al. 2020). The reduced amylase activity observed in the CS6 supports the hypothesis of inhibited amylolytic function. In CS6, which is a lower-fat diet compared with CS11 and CS16, phenolic compounds were likely more bioavailable and able to interact directly with rumen microbes and enzymes such as amylase, thereby limiting starch degradation (Sun et al. 2019). A wide variety of plant-derived feed additives have been investigated as rumen habitat modifiers due to their ability to selectively influence rumen microbial activity and fermentation patterns (Tsiplakou et al. 2023). Essential oils, which are secondary metabolites extracted from the volatile fraction of plants, have been reported to exert inhibitory effects on ruminal microbial activity through their action on functional bacterial groups involved in deamination and starch utilization, including hyper-ammonia-producing bacteria such as *Prevotella* spp. and *Ruminobacter amylophilus* (Tsiplakou et al. 2023). Therefore, aromatic plants and essential oils rich in phenolic compounds can theoretically selectively inhibit starch-degrading microbes without completely suppressing overall rumen fermentation (Calsamiglia et al. 2007). In addition, starch-degrading microbes may have reduced competition for fiber, potentially promoting fiber-degraders to grow. In the higher fat content diets (CS11 and CS16), fats may coat feed particles or interact with phenols, reducing their antimicrobial activity and allowing starch-degrading microbes to thrive. This could potentially promote starch-degrading microbes. Dietary fats can shift rumen microbial populations, potentially promoting microbes less sensitive to phenols or more efficient at starch digestion despite phenolic presence, although excessive fat may also suppress fiber degraders such as *Ruminococcus* (Maia et al. 2007). Considering the abovementioned, in CS6, phenolic compounds may have a

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

stronger inhibitory effect on starch-degrading while supporting fiber-degrading microbes, whereas in CS11 and CS16, the higher fat content levels likely masked the phenolic effect, favoring starch-utilizing microbes and suppressing fiber breakdown. In addition, the lowest VFA concentrations were observed in the CS6, potentially due to enhanced phenolic bioavailability and reduced starch fermentation, while CS11 and CS16, with higher fat and greater *Camelina* seed inclusion levels, supported higher VFA production than both CS6 and the Control. Overall, these findings highlight the importance of considering both phenolic compounds and dietary fat when evaluating the effects of *Camelina* seeds on rumen fermentation and microbial populations.

Another key finding was the increased abundance of *Selenomonas*, known for its ability to utilize lactate and produce propionate, in CS16, which assists in maintaining rumen pH stability by preventing lactate accumulation (Millen et al. 2016). This result is consistent with our previous findings from targeted RT-qPCR analysis of *Selenomonas ruminantium*, as reported in Christodoulou et al. (2023). The positive correlation of *Selenomonas* with acetic and propionic acids further supports its potential involvement in carbohydrate fermentation and VFA production under this dietary treatment. The increased abundance of *Selenomonas* in CS16 could suggest its adaptability to different ruminal niches. It may be attributed to the nutrient profile of CS, particularly its PUFA and fermentable substrates. The presence of PUFA, especially omega-3 fatty acids, could modulate rumen microbial populations by favoring propionate-producing pathways, while potentially reducing acetate production, as in previous studies with dietary flaxseed inclusion (Vargas et al. 2020; Huang et al. 2021). This shift may also have implications for methane mitigation, as propionate production competes with methanogenesis for hydrogen, thereby reducing methane emissions (Wang et al. 2023). This is further supported by the decreased (numerically) abundance of *Methanobrevibacter* at higher *Camelina* levels, although the studied region (V3–V4) is not representative of these taxa (Zhou et al. 2021). In compliance with the present findings, reported reductions in methanogenic archaea with increasing CS inclusion levels were also observed through the targeted RT-qPCR analysis in Christodoulou et al. (2023), suggesting that PUFA-rich diets potentially suppress methanogen-associated communities, despite differences in analytical resolution between RT-qPCR and 16S amplicon sequencing. In addition, the reduced acetic: propionic ratio in CS11 and CS16 highlights a shift toward pathways favoring propionate production, and competing with methanogenesis for hydrogen utilization (Hook et al. 2010). Moreover, the negative correlation with *Methanobrevibacter* could also point to competition for hydrogen between methanogenesis and propionate-producing pathways. In accordance with this finding, the *Succinivibrionaceae* family, which was in higher abundances in CS16, could also be linked with the higher propionate production in these treatments (Ren et al. 2019; Han et al. 2021).

The different responses between rumen fluid and solid microbiota are critical to understanding rumen functionality. The results of the present study highlight the complex microbial shifts occurring in both rumen fluid and solid particles in response to varying levels of *C. sativa* seed inclusion. Considering the abovementioned, the present 16S amplicon sequencing-based findings extend the observations of Christodoulou et al. (2023) by demonstrating that PUFA-rich CS inclusion induces consistent shifts in rumen microbial communities across multiple taxonomic levels, while also revealing additional taxa and patterns that are not detectable using targeted approaches. The rumen fluid microbiota, which helps break down rapidly fermentable nutrients, showed reduced diversity and shifts in composition. In contrast, the fiber-attached microbes in the solid phase had a more stable diversity, but their composition changed in ways that suggest a shift in function. Holistically, profiling both compartments with 16S amplicon sequencing provides valuable insights into how dietary modifications influence microbial ecology and rumen function. Nevertheless, dairy ewes' performance in this trial was not significantly affected (Christodoulou et al. 2021). This could suggest that despite the microbial shifts and the different fermentation patterns, ewes could efficiently digest their feed and extract sufficient nutrients for maintenance and production. Overall, additional key parameters, such as pH and ammonia, should be considered to better link the rumen microbiome with rumen function and metabolism.

Conclusion

This study highlights that the complex interaction between fat content and plant compounds such as glucosinolates in the diet can modify the rumen microbiome. Inclusion of *C. sativa* seeds at a lower fat content level (CS6) appeared to favor fiber-digesting bacteria like *Fibrobacter*, likely due to the enhanced activity of bioavailable phenolics suppressing starch-degrading microbes. In contrast, higher fat content diets (CS11 and CS16) reduced the microbiota diversity (for the rumen fluid compartment) and showed potentially reduced phenolic impact, most likely by altering their availability or through direct fat effects, favoring amylolytic populations. Therefore, balancing both secondary metabolites and nutrients in ruminant diets is essential to optimize rumen fermentation and microbial function.

Acknowledgments

The authors gratefully acknowledge the technical and research services staff within the Research Institute of Animal Science, ELGO DIMITRA, Paralimni, Giannitsa, for the technical support and assistance in this work.

Author contributions

Christos Christodoulou (Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft, Writing—review & editing), Alexandros Mavrommatis (Data curation, Investigation, Methodology, Writing—review & editing), Marco Severgnini (Data curation, Formal analysis, Software, Visualization, Writing—review & editing), Paola Cremonesi (Writing—review & editing), Bianca Castiglioni (Writing—review & editing), Panagiota Kyriakaki (Investigation), Rafaela Andreaki (Investigation), Basiliki Kotsampasi (Investigation), and Eleni Tsiplakou (Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing—review & editing)

Supplementary data

Supplementary data is available at *Journal of Animal Science* online.

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

Funding

None declared.

Conflict of interest statement: The authors declare no real or perceived conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abuelfatah K, Zuki AB, Goh YM, Sazili AQ, Abubakr A. 2016. Effects of feeding whole linseed on ruminal fatty acid composition and microbial population in goats. Anim Nutr. 2:323–328. https://doi.org/10.1016/j.aninu.2016.10.004

Bayat AR et al. 2015. Effect of camelina oil or live yeasts (Saccharomyces cerevisiae) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. J Dairy Sci. 98:3166–3181. https://doi.org/10.3168/jds.2014-7976

Betancur-Murillo CL, Aguilar-Marín SB, Jovel J. 2022. Prevotella: a key player in ruminal metabolism. Microorganisms. 11:1. https://doi.org/10.3390/microorganisms11010001

Brandao VLN et al. 2018. Effect of replacing calcium salts of palm oil with camelina seed at 2 dietary ether extract levels on digestion, ruminal fermentation, and nutrient flow in a dual-flow continuous culture system. J Dairy Sci. 101:5046–5059. https://doi.org/10.3168/jds.2017-13558

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J Dairy Sci. 90:2580–2595. https://doi.org/10.3168/jds.2006-644

Caporaso JG et al. 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

Cheng Z et al. 2022. Effects of supplementation of sodium acetate on rumen fermentation and microbiota in postpartum dairy cows. Front Microbiol. 13:1053503. https://doi.org/10.3389/fmicb.2022.1053503

Christodoulou C et al. 2023. Inclusion of camelina sativa seeds in ewes' diet modifies rumen microbiota. Animals (Basel). 13: 377. https://doi.org/10.3390/ani13030377

Christodoulou C et al. 2021. Assessing the optimum level of supplementation with camelina seeds in ewes' diets to improve milk quality. Foods. 10:2076.https://doi.org/10.3390/foods10092076

Cieslak A et al. 2013. Camelina sativa affects the fatty acid contents in M. longissimus muscle of lambs. Euro J Lipid Sci Tech. 115:1258–1265. https://doi.org/10.1002/ejlt.201200119

Dai X et al. 2017. Camelina seed supplementation at two dietary fat levels change ruminal bacterial community composition in a dual-flow continuous culture system. Front Microbiol. 8:2147. https://doi.org/10.3389/fmicb.2017.02147

Edgar RC. 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv: 081257. https://doi.org/10.1101/081257

Giuberti G, Rocchetti G, Lucini L. 2020. Interactions between phenolic compounds, amylolytic enzymes and starch: an updated overview. Curr Opin Food Sci. 31:102–113. https://doi.org/10.1016/j.cofs.2020.04.003

Griswold KE, White BA, Mackie RI. 1999. Diversity of extracellular proteolytic activities among *Prevotella* species from the rumen. Curr Microbiol. 39:187–194. https://doi.org/10.1007/s002849900443

Han X et al. 2021. A metagenomic insight into the hindgut microbiota and their metabolites for dairy goats fed different rumen degradable starch. Front Microbiol. 12:651631. https://doi.org/10.3389/fmicb.2021.651631

Henderson G et al. 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

Hook SE, Wright AD, McBride BW. 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea. 2010:945785. https://doi.org/10.1155/2010/945785

Huang G et al. 2021. Effect of whole or ground flaxseed supplementation on fatty acid profile, fermentation, and bacterial composition in rumen of dairy cows. Front Microbiol. 12:760528. https://doi.org/10.3389/fmicb.2021.760528

Hurtaud C, Peyraud JL. 2007. Effects of feeding camelina (seeds or meal) on milk fatty acid composition and butter spreadability. J Dairy Sci. 90:5134–5145. https://doi.org/10.3168/jds.2007-0031

Huws SA et al. 2018. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. Front Microbiol. 9:2161. https://doi.org/10.3389/fmicb.2018.02161

Jami E, Mizrahi I. 2012. Composition and similarity of bovine rumen microbiota across individual animals. PLoS One. 7:e33306. https://doi.org/10.1371/journal.pone.0033306

Kaminsky RA et al. 2023. Rumen *lachnospiraceae* isolate NK3A20 exhibits metabolic flexibility in response to substrate and coculture with a methanogen. Appl Environ Microbiol. 89:e00634-00623. https://doi.org/10.1128/aem.00634-23

Klindworth A et al. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41:e1. https://doi.org/10.1093/nar/gks808

Lee PC, Lee WG, Kwon S, Lee SY, Chang HN. 1999. Succinic acid production by *Anaerobiospirillum succiniciproducens*: effects of the H_2/CO_2 supply and glucose concentration. Enzyme Microb Technol. 24:549–554. https://doi.org/10.1016/S0141-0229(98)00156-2

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J. 5:169–172. https://doi.org/10.1038/ismej.2010.133

Maia MRG, Chaudhary LC, Figueres L, Wallace RJ. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek. 91:303–314. https://doi.org/10.1007/s10482-006-9118-2

Martin, C, and Michalet-Doreau B.. 1995. Variations in mass and enzyme activity of rumen microorganisms: effect of barley and buffer supplements. J Sci Food Agric. 67:407–413. https://doi.org/10.1002/jsfa.2740670319

Masella AP, Bartram RJ, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics. 13:31. https://doi.org/10.1186/1471-2105-13-31

Mavrommatis A et al. 2025. The impact of probiotic live yeast in a barley grain-based diet on rumen microbial communities,

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026

fermentation, and histology of artificially reared lambs. Anim Feed Sci Technol. 322:116269. https://doi.org/10.1016/j.anifeedsci.2025.116269

Mavrommatis A, Skliros D, Flemetakis E, Tsiplakou E. 2021. Changes in the rumen bacteriome structure and enzymatic activities of goats in response to dietary supplementation with *Schizochytrium* spp. Microorganisms. 9:1528.

Millen DD et al. 2016. Ruminal acidosis. In: DD Millen, M De Beni Arrigoni and RD Lauritano Pacheco, editors. Rumenology. Springer International Publishing. p. 127–156.

Morgavi DP et al. 2015. Rumen microbial communities influence metabolic phenotypes in lambs. Front Microbiol. 6:1060. https://doi.org/10.3389/fmicb.2015.01060

Muizelaar WP et al. 2020. Rumen fluid sampling via oral stomach tubing method. In: G Viereck, B Kuhla, S Danesh Mesgaran, editors. Methods in cattle physiology and behaviour research – recommendations from the SmartCow consortium. PUBLISSO. p. 1–6.

Nagaraja TG. 2016. Microbiology of the rumen. In: DD Millen, M De Beni Arrigoni, RD Lauritano Pacheco, editors. Rumenology. Springer International Publishing. p. 39–61.

Owens FN, Basalan M. 2016. Ruminal fermentation. In: DD Millen, M De Beni Arrigoni, RD Lauritano Pacheco, editors. Rumenology. Springer International Publishing. p. 63–102.

Palevich N et al. 2019. Comparative genomics of rumen *Butyrivibrio* spp. uncovers a continuum of polysaccharide-degrading capabilities. Appl Environ Microbiol. 86:e01993-01919. doi: https://doi.org/10.1128/AEM.01993-19

Patra AK. 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: a meta-analysis. Livest Sci. 155:244–254. https://doi.org/10.1016/j.livsci.2013.05.023

Poretsky R, Rodriguez RL, Luo C, Tsementzi D, Konstantinidis KT. 2014. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One. 9:e93827. https://doi.org/10.1371/journal.pone.0093827

Quast C et al. 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

Ramos-Morales E et al. 2014. Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats. Anim Feed Sci Technol. 198:57–66. https://doi.org/10.1016/j.anifeedsci.2014.09.016

Ren H et al. 2019. Specific enrichment of microbes and increased ruminal propionate production: the potential mechanism underlying the high energy efficiency of holstein heifers fed steam-flaked corn. AMB Express. 9:209. https://doi.org/10.1186/s13568-019-0937-8

Riaz R, Ahmed I, Sizmaz O, Ahsan U. 2022. Use of camelina sativa and by-products in diets for dairy cows: a review. Animals. 12:1082. https://doi.org/10.3390/ani12091082

Russell JB, Rychlik JL. 2001. Factors that alter rumen microbial ecology. Science. 292:1119–1122. https://doi.org/10.1126/science.1058830

Sun L, Warren FJ, Gidley MJ. 2019. Natural products for glycaemic control: polyphenols as inhibitors of alpha-amylase. Trends Food Sci Technol. 91:262–273. https://doi.org/10.1016/j.tifs.2019.07.009

Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. 2021. Prevotella diversity, niches and interactions with the human host. Nat Rev Microbiol. 19:585–599. https://doi.org/10.1038/s41579-021-00559-y

Tsiplakou EA, Mavrommatis AI, Gelasakis AI, Kalogianni AI, Simitzis PE. 2023. Effect of phytochemical feed additives on health status, milk yield, and quality characteristics in ruminants. In: G Arsenos, I Giannenas, editors. Sustainable use of feed additives in livestock: novel ways for animal production. Springer International Publishing. p. 641–663.

Vargas JE et al. 2020. Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. Sci Rep. 10:1613. https://doi.org/10.1038/s41598-020-58401-z

Wang K, Xiong B, Zhao X. 2023. Could propionate formation be used to reduce enteric methane emission in ruminants? Sci Total Environ. 855:158867. https://doi.org/10.1016/j.scitotenv.2022.158867

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 73:5261–5267. doi: https://doi.org/10.1128/AEM.00062-07

Whittaker RH. 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecol Monogr. 30:279–338. https://doi.org/10.2307/1943563

Zhou M et al. 2021. Accessing dietary effects on the rumen microbiome: Different sequencing methods tell different stories. Vet Sci. 8:138. https://doi.org/10.3390/vetsci8070138

Downloaded from https://academic.oup.com/jas/article/doi/10.1093/jas/skag033/8483036 by guest on 29 May 2026