Long-term effects of dietary supplementation with olive oil and hydrogenated vegetable oil on the rumen microbiome of dairy cows

Nathaly Cancino-Padilla¹, Natalia Catalán², Karen Siu-Ting³, Christopher J. Creevey³, Sharon A. Huws³, Jaime Romero² and Einar Vargas-Bello-Pérez⁴.¹*

¹Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Avda. Vicuña Mackenna 4860, Santiago 6904411, Chile; nathaly.cancino@gmail.com

²Laboratorio de Biotecnología de Alimentos, Unidad de Alimentos, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, El Líbano 5524, Macul, Santiago 7830490, Chile; nataliabcatalan@gmail.com

³Institute for Global Food Security, School of Biological Sciences, Queen’s University of Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, UK; agalychnica@gmail.com (K.S.-T.); chris.creevey@qub.ac.uk (C.J.C.); s.huws@qub.ac.uk (S.A.H.)

⁴Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 3, DK-1870 Frederiksberg C, Denmark

*Correspondence: jromero@inta.uchile.cl (J.R.); evargasb@sund.ku.dk (E.V.-B.-P.)
### Supplementary material

Table S1. Most Prevalent Taxa: Phylum level.
Mean relative abundance (%) at different time point of the most prevalent phylum from cows fed with control, HVO and OO treatments

| Phylum      | Treatments | Control¹ | HVO² | OO³ | SEM⁴ | P-value |
|-------------|------------|----------|------|-----|------|---------|
| **21 days** |            |          |      |     |      |         |
| Firmicutes  |            | 60.16    | 82.75| 72.83| 6.54 | 0.10    |
| Bacteroidetes|           | 37.84    | 15.03| 23.78| 6.64 | 0.10    |
| Actinobacteria|          | 0.80     | 0.76 | 2.20 | 0.47 | 0.13    |
| Proteobacteria|          | 0.29     | 0.40 | 0.52 | 0.07 | 0.25    |
| Others      |            | 0.91     | 1.06 | 0.67 | 0.12 | 0.37    |
| **42 days** |            |          |      |     |      |         |
| Firmicutes  |            | 65.97    | 74.68| 64.82| 3.11 | 0.63    |
| Bacteroidetes|           | 31.60    | 22.25| 32.05| 3.19 | 0.54    |
| Actinobacteria|          | 1.02     | 1.44 | 1.71 | 0.20 | 0.82    |
| Proteobacteria|          | 0.75     | 0.99 | 1.03 | 0.09 | 0.56    |
| Others      |            | 0.66     | 0.63 | 0.39 | 0.08 | 0.54    |
| **63 days** |            |          |      |     |      |         |
| Firmicutes  |            | 81.19    | 84.92| 87.42| 1.81 | 0.59    |
| Bacteroidetes|           | 16.39    | 12.61| 9.612| 1.96 | 0.51    |
| Actinobacteria|          | 1.56     | 1.15 | 2.19 | 0.30 | 0.35    |
| Proteobacteria|          | 0.29     | 0.35 | 0.44 | 0.05 | 0.35    |
| Others      |            | 0.57     | 0.97 | 0.34 | 0.18 | 0.07    |

¹Control, no fat supplement; ²HVO, supplemented with 3% DM hydrogenated vegetable oil; ³OO, supplemented with 3% DM olive oil; ⁴SEM: standard error of the mean and values are LSM and pooled SEM; ⁵Others, less abundant Phylum (relative abundance ≤ 0.5%). Data were analyzed by one-way ANOVA and means were compared by Kruskal-Wallis test. Results were declared significant at P<0.05.
Table S2. Most Prevalent Taxa: Family level.
Mean relative abundance (%) at different time point of the most prevalent families from cows fed with control, HVO and OO treatments

| Family               | Treatments       | Control³ | HVO²  | OO¹  | SEM⁴ | P-value |
|----------------------|------------------|----------|-------|------|------|---------|
|                      |                  |          |       |      |      |         |
| 21 days              |                  |          |       |      |      |         |
| Ruminococcaceae      | 21.15            | 25.31    | 25.52 | 1.42 | 0.58 |
| Lachnospiraceae      | 29.12            | 23.96    | 35.39 | 3.30 | 0.15 |
| Prevotellaceae       | 12.37            | 8.93     | 7.58  | 1.43 | 0.54 |
| Acidaminococcaceae   | 14.70            | 16.88    | 12.69 | 1.21 | 0.71 |
| Others               | 22.67            | 24.92    | 18.82 | 1.78 | 0.05 |
| 42 days              |                  |          |       |      |      |         |
| Ruminococcaceae      | 17.59            | 21.90    | 16.86 | 1.57 | 0.63 |
| Lachnospiraceae      | 19.05            | 22.35    | 21.39 | 0.98 | 0.84 |
| Prevotellaceae       | 26.80            | 18.15    | 28.66 | 3.24 | 0.54 |
| Acidaminococcaceae   | 17.01            | 16.99    | 13.14 | 1.29 | 0.63 |
| Others               | 19.55            | 20.62    | 19.95 | 0.31 | 0.99 |
| 63 days              |                  |          |       |      |      |         |
| Ruminococcaceae      | 17.42            | 29.60    | 22.84 | 3.52 | 0.06 |
| Lachnospiraceae      | 12.74            | 19.05    | 23.09 | 3.01 | 0.15 |
| Prevotellaceae       | 30.30            | 9.78     | 18.54 | 5.95 | 0.12 |
| Acidaminococcaceae   | 16.24            | 18.41    | 10.41 | 2.39 | 0.40 |
| Others               | 23.29            | 23.17    | 25.13 | 0.63 | 0.75 |

³Control, no fat supplement; ²HVO, supplemented with 3% DM hydrogenated vegetable oil; ¹OO, supplemented with 3% DM olive oil; ⁴SEM: standard error of the mean standard error of the mean and values are LSM and pooled SEM. ⁵Others' refers to the less abundant Phylum (relative abundance ≤ 0.5%). Data were analyzed by one-way ANOVA and means were compared by Kruskal-Wallis test. Results were declared significant at P<0.05.

Table S3. Most Prevalent Taxa: Genus level.
Mean relative abundance (%) at different time point of the most prevalent genus from cows fed with control, HVO and OO treatments

| Treatments | Genus                      | 1Control | 2HVO | 3OO | SEM   | P-value |
|------------|----------------------------|----------|------|-----|-------|---------|
| 21 days    | Prevotella_1               | 29.15    | 8.63 | 17.97 | 5.93 | 0.20    |
|            | Succiniclasticum           | 19.13    | 21.44 | 13.12 | 2.48 | 0.45    |
|            | Ruminococcaceae_NK4A214_group | 9.16    | 13.65 | 11.65 | 1.30 | 0.20    |
|            | Christensenellaceae_R-7_group | 7.83    | 10.46 | 6.57  | 1.15 | 0.27    |
|            | Ruminococcaceae_UCG-014    | 5.31     | 9.26  | 6.19  | 1.20 | 0.25    |
|            | Lachnospiraceae_NK3A20_group | 3.03    | 4.86  | 6.12  | 0.89 | 0.33    |
|            | Others                     | 0.262    | 0.315 | 0.382 | 0.035 | 0.001   |
| 42 days    | Prevotella_1               | 24.95    | 16.28 | 27.10 | 3.31 | 0.54    |
|            | Succiniclasticum           | 21.07    | 20.87 | 16.23 | 1.58 | 0.54    |
|            | Ruminococcaceae_NK4A214_group | 7.89    | 10.11 | 8.28  | 0.69 | 0.77    |
|            | Christensenellaceae_R-7_group | 7.25    | 8.00  | 4.95  | 0.92 | 0.71    |
|            | Ruminococcaceae_UCG-014    | 4.08     | 8.55  | 4.30  | 1.46 | 0.45    |
|            | Lachnospiraceae_NK3A20_group | 4.61    | 6.28  | 5.25  | 0.49 | 0.91    |
|            | Others                     | 0.298    | 0.298 | 0.338 | 0.013 | 0.82    |
| 63 days    | Prevotella_1               | 12.09    | 8.42  | 6.99  | 1.52 | 0.54    |
|            | Succiniclasticum           | 18.74    | 21.09 | 17.14 | 1.15 | 0.59    |
|            | Ruminococcaceae_NK4A214_group | 11.35   | 12.04 | 15.74 | 1.36 | 0.51    |
|            | Christensenellaceae_R-7_group | 10.95   | 12.12 | 6.43  | 1.73 | 0.09    |
|            | Ruminococcaceae_UCG-014    | 4.66     | 4.75  | 5.41  | 0.24 | 0.91    |
|            | Lachnospiraceae_NK3A20_group | 7.82    | 6.25  | 11.56 | 1.58 | 0.23    |
|            | Others                     | 0.343    | 0.350 | 0.364 | 0.006 | 0.63    |

1Control, no fat supplement; 2HVO, supplemented with 3% DM hydrogenated vegetable oil; 3OO, supplemented with 3% DM olive oil; 4SEM: standard error of the mean standard error of the mean and values are LSM and pooled SEM. 5Others’ refers to the less abundant Phylum (relative abundance ≤ 0.5%). Data were analyzed by one-way ANOVA and means were compared by Kruskal-Wallis test. Results were declared significant at P<0.05.

Table S4. PERMANOVA (Weighted) results.
PERMANOVA (Weighted) analysis of the effect of dietary treatments on rumen bacterial diversity.
### Table S5. PERMANOVA (Weighted) results.
**PERMANOVA (UnWeighted) analysis of the effect of dietary treatments on rumen bacterial diversity**

| Treatment    | Period | SumsOfSqS | MeanSqs | F.Model | R2   | Pr(>F) |
|--------------|--------|-----------|---------|---------|------|--------|
| Control vs. HVO | 21 days | 0.063     | 0.063   | 2.812   | 0.319| 0.052  |
| Control vs. HVO | 42 days | 0.023     | 0.023   | 0.450   | 0.069| 0.760  |
| Control vs. HVO | 63 days | 0.006     | 0.006   | 0.598   | 0.091| 0.658  |
| Control vs. OO | 21 days | 0.022     | 0.022   | 1.717   | 0.222| 0.229  |
| Control vs. OO | 42 days | 0.017     | 0.018   | 1.247   | 0.172| 0.320  |
| Control vs. OO | 63 days | 0.015     | 0.015   | 1.505   | 0.200| 0.255  |

SumOfSqs, sum of squares; MeanSqs, mean sum of squares; F, F value by permutation; Statistical significance was declared at P<0.05; P-value based on 999 permutations.

### Table S6. Predicted Functions in Control Diet.
**Predicted functions with significant variations in control diet**

| Predicted functions                                  | P-value |
|------------------------------------------------------|---------|
|                                                      | 21 days | 42 days | 63 days |
| ABC transporters                                     | 0.81    | 0.99    | < 0.01  |
| DNA repair and recombination proteins                 | < 0.01  | 0.99    | < 0.01  |
| Pores ion channels                                   | 0.74    | 0.25    | < 0.01  |
| Protein kinases                                      | 0.24    | 0.04    | 0.39    |
| Purine metabolism                                    | 0.01    | 0.97    | 0.12    |
| Pyrimidine metabolism                                | 0.04    | 0.83    | 0.99    |
| Ribosome                                             | 0.03    | 0.77    | 0.73    |
| Transcription factors                                | 0.96    | 0.88    | < 0.01  |
| Transporters                                         | 0.99    | 0.46    | < 0.01  |
| Two-component system                                 | < 0.01  | < 0.01  | < 0.01  |

Data were analyzed by multiple group ANOVA and Dunnett's multiple comparisons test. Results were declared significant at P<0.01.

### Table S7. Predicted Functions in OO Diet.
### Predicted functions with significant variations in olive oil diet

| Predicted functions                                             | 21 days | 42 days | 63 days |
|----------------------------------------------------------------|---------|---------|---------|
| ABC transporters                                              | 0.93    | < 0.01  | 0.86    |
| Alanine, aspartate and glutamate metabolism                    | 0.99    | 0.01    | 0.21    |
| Amino acid related enzymes                                     | 0.92    | < 0.01  | 0.05    |
| Amino sugar and nucleotide sugar metabolism                    | 0.99    | < 0.01  | 0.25    |
| Aminoacyl-tRNA biosynthesis                                    | 0.88    | 0.01    | 0.29    |
| Arginine and proline metabolism                                | 0.98    | 0.02    | 0.28    |
| Bacterial motility proteins                                    | 0.97    | 0.01    | 0.35    |
| Carbon fixation pathways in prokaryotes                        | > 0.99  | 0.04    | 0.34    |
| Chaperones and folding catalysts                               | 0.99    | 0.02    | 0.15    |
| Chromosome                                                     | 0.79    | < 0.01  | 0.22    |
| DNA repair and recombination proteins                           | 0.34    | < 0.01  | 0.02    |
| General function prediction only                               | 0.77    | < 0.01  | 0.15    |
| Glycine, serine and threonine metabolism                       | 0.97    | 0.04    | 0.22    |
| Methane metabolism                                             | 0.99    | < 0.01  | 0.26    |
| Other ion-coupled transporters                                 | 0.70    | 0.01    | 0.72    |
| Peptidases                                                     | 0.89    | < 0.01  | 0.04    |
| Pores ion channels                                             | 0.84    | 0.24    | < 0.01  |
| Protein kinases                                                | 0.37    | < 0.01  | 0.03    |
| Purine metabolism                                              | 0.61    | < 0.01  | 0.02    |
| Pyrimidine metabolism                                          | 0.89    | < 0.01  | 0.01    |
| Pyruvate metabolism                                            | 0.98    | 0.03    | 0.46    |
| Ribosome                                                       | 0.84    | < 0.01  | 0.01    |
| Ribosome Biogenesis                                            | 0.95    | < 0.01  | 0.06    |
| Starch and sucrose metabolism                                  | 0.99    | < 0.01  | 0.29    |
| Transcription factors                                          | 0.99    | < 0.01  | 0.92    |
| Transcription machinery                                        | 0.97    | < 0.01  | 0.03    |
| Transporters                                                   | 0.97    | < 0.01  | 0.14    |
| Two-component system                                           | 0.015   | < 0.01  | < 0.01  |

Data were analyzed by multiple group ANOVA and Dunnett’s multiple comparisons test. Results were declared significant at P<0.01.

Table S8. Predicted Functions in HVO Diet.
Predicted functions with significant variations in hydrogenated vegetable oil diet

| Predicted functions                                      | 21 days | 42 days | 63 days |
|---------------------------------------------------------|---------|---------|---------|
| ABC transporters                                        | 0.44    | < 0.01  | 0.22    |
| Alanine, aspartate and glutamate metabolism             | 0.09    | 0.02    | 0.06    |
| Amino acid related enzymes                              | < 0.01  | < 0.01  | < 0.01  |
| Amino sugar and nucleotide sugar metabolism             | 0.02    | < 0.01  | 0.01    |
| Aminoacyl-tRNA biosynthesis                             | 0.09    | 0.01    | 0.05    |
| Arginine and proline metabolism                         | 0.19    | 0.03    | 0.13    |
| Chaperones and folding catalysts                        | 0.05    | 0.02    | 0.03    |
| Chromosome                                              | 0.040   | < 0.01  | 0.02    |
| DNA repair and recombination proteins                    | < 0.01  | < 0.01  | < 0.01  |
| DNA replication proteins                                | 0.22    | 0.05    | 0.12    |
| General function prediction only                        | 0.04    | < 0.01  | 0.01    |
| Methane metabolism                                      | 0.17    | 0.022   | 0.12    |
| Other ion-coupled transporters                          | 0.17    | 0.02    | 0.09    |
| Peptidases                                              | < 0.01  | < 0.01  | < 0.01  |
| Pores ion channels                                      | < 0.01  | 0.02    | < 0.01  |
| Protein kinases                                         | 0.03    | < 0.01  | 0.01    |
| Purine metabolism                                       | < 0.01  | < 0.01  | < 0.01  |
| Pyrimidine metabolism                                   | < 0.01  | < 0.01  | < 0.01  |
| Ribosome                                                | < 0.01  | < 0.01  | < 0.01  |
| Ribosome Biogenesis                                     | < 0.01  | < 0.01  | < 0.01  |
| Starch and sucrose metabolism                           | 0.02    | < 0.01  | 0.01    |
| Transcription factors                                   | 0.18    | < 0.01  | 0.19    |
| Transcription machinery                                 | 0.02    | 0.01    | 0.01    |
| Transporters                                            | < 0.01  | < 0.01  | < 0.01  |
| Two-component system                                    | < 0.01  | < 0.01  | < 0.01  |

Data were analyzed by multiple group ANOVA and Dunnett’s multiple comparisons test. Results were declared significant at P<0.01.
| Level 1                              | Level 2                          | Level 3                                      | Database       |
|-------------------------------------|----------------------------------|----------------------------------------------|----------------|
| Environmental Information Processing | Membrane transport               | ABC transporters                            | PATHWAY        |
| Signal transduction                 | Two-component system             |                                              | PATHWAY        |
| Genetic Information Processing      | Translation                      | Aminoacyl-tRNA biosynthesis                  | PATHWAY        |
|                                    | Ribosome                         |                                              | PATHWAY        |
|                                    | Transcription                    | Transcription factors                        | PATHWAY        |
| Metabolism                          | Amino Acid Metabolism            | Alanine, aspartate and glutamate metabolism  | PATHWAY        |
|                                     |                                  | Glycine, serine and threonine metabolism     | PATHWAY        |
|                                     |                                  | Arginine and proline metabolism              | PATHWAY        |
| Carbohydrate Metabolism            | Amino sugar and nucleotide sugar metabolism |                                              | PATHWAY        |
| Energy Metabolism                   | Pyruvate metabolism              |                                              | PATHWAY        |
|                                    | Starch and sucrose metabolism    |                                              | PATHWAY        |
| Nucleotide Metabolism              | Carbon fixation pathways in prokaryotes |                                              | PATHWAY        |
|                                    | Methane metabolism               |                                              | PATHWAY        |
| Genes and proteins                  | DNA repair and recombination proteins |                                              | BRITE          |
|                                    | Chaperones and folding catalysts |                                              | BRITE          |
|                                    | Chromosome                       |                                              | BRITE          |
|                                    | Ribosome Biogenesis               |                                              | BRITE          |
|                                    | Transcription machinery          |                                              | BRITE          |
|                                    | DNA replication proteins         |                                              | BRITE          |
| Signaling and Cellular Processes    | Transports                       |                                              | BRITE          |
|                                    | Bacterial motility proteins      |                                              | BRITE          |
| Metabolism                          | Protein kinases                  |                                              | BRITE          |
|                                    | Amino acid related enzymes       |                                              | BRITE          |
|                                    | Peptidases                       |                                              | BRITE          |
| Unclassified                        | Pores ion channels               |                                              | BRITE          |
|                                    | Other ion-coupled transporters   |                                              | BRITE          |
| Poorly Characterized                | General function prediction only |                                              | BRITE          |