**Supplemental Table 1.** List of primers used in real-time assay

|  |  |  |  |
| --- | --- | --- | --- |
| **Target** | **5' – sequence1** | **Ta2** | **Reference3** |
| *Megasphaera elsdenii* | f-GACCGAAACTGCGATGCTAGA | 60 | (46) |
|  | r-CGCCTCAGCGTCAGTTGTC |  |  |
| *Ruminococcus albus* | f-CCCTAAAAGCAGTCTTAGTTCG | 61 | (47) |
|  | r-CCTCCTTGCGGTTAGAACA |  |  |
| *Selenomonas ruminantium* group | f-CAATAAGCATTCCGCCTGGG | 61 | (6) |
|  | r-TTCACTCAATGTCAAGCCCTGG |  |  |
| *Fibrobacter succinogenes* | f-GCGGGTAGCAAACAGGATTAGA | 60 | (6) |
|  | r-CCCCCGGACACCCAGTAT |  |  |
| *Streptococcus bovis* | f-TTCCTAGAGATAGGAAGTTTCTTCGG | 60 | (6) |
|  | r-ATGATGGCAACTAACAATAGGGGT |  |  |
| *Prevotella bryantii* | f-AGCGCAGGCCGTTTGG | 61 | (6) |
|  | r-GCTTCCTGTGCACTCAAGTCTGAC |  |  |
| *Prevotella ruminicola* | f-GAAAGTCGGATTAATGCTCTATGTTG | 58 | (6) |
|  | r-CATCCTATAGCGGTAAACCTTTGG |  |  |
| *B. fibrisolvens/Pseudobutyrivibrio* | f-GCCTCAGCGTCAGTAATCG | 65 | (14) |
|  | r-GGAGCGTAGGCGGTTTTAC |  |  |
| *Butyrivibrio hungatei* | f-AGGGTAATGCCTGTAGCTC | 55 | (14) |
|  | r-TCACCCTCGCGGGAT |  |  |
| Fungi | f-GAGGAAGTAAAAGTCGTAACAAGGTTTC | 60 | (48) |
|  | r-CAAATTCACAAAGGGTAGGATGATT |  |  |
| Ciliate protozoa | f-GCTTTCGWTGGTAGTGTATT | 54 | (49) |
|  | r-CTTGCCCTCYAATCGTWCT |  |  |
| Total bacteria | f-CGGCAACGAGCGCAACCC | 60 | (48) |
|  | r-CCATTGTAGCACGTGTGTAGCC |  |  |

1Selected forward (f) and reverse (r) primers for validated for genes encoding 16S ribosomal RNA (bacterial species and fungi), and for the 18S ribosomal RNA gene (protozoa).

2Annealing temperature.

3Reference number for the source of primers.

**Supplemental Table 2.** Abundance of select ruminal microorganisms during induction of and recovery from milk fat depression.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatment means1,2 | | |  | *P-*value3 | | |
| Item1 | Control | Induction | Recovery | SEM | Trt | Time | Trt x time |
|  | % of total bacteria4 | | |  |  |  |  |
| *Megasphaera elsdenii* | 0.00029 | 0.0020 | 0.00064 | 0.002 | 0.02 | 0.59 | 0.01 |
| *Ruminococcus albus* | 0.117 | 0.087 | 0.109 | 0.018 | 0.24 | 0.02 | <0.01 |
| *Selenomonas ruminantium* | 0.13 | 0.19 | 0.13 | 0.016 | <0.01 | 0.34 | <0.01 |
| *Fibrobacter succinogenes* | 0.967 | 0.027 | 0.337 | 0.13 | <0.001 | 0.13 | <0.001 |
| *Streptococcus bovis* | 0.0049 | 0.014 | 0.0042 | 0.007 | <0.001 | 0.04 | <0.001 |
| *Prevotella bryantii* | 0.091 | 0.071 | 0.044 | 0.034 | <0.01 | 0.18 | <0.01 |
| *Prevotella ruminicola* | 0.84 | 0.69*I* | 0.98r | 0.08 | <0.01 | 0.10 | 0.59 |
| *B. fibrisolvens/Pseudobutyrivibrio* | 0.98 | 0.53 | 0.68 | 0.17 | <0.01 | 0.15 | <0.001 |
| *Butyrivibrio hungatei* | 0.035 | 0.039 | 0.042 | 0.010 | 0.52 | 0.33 | 0.67 |
| Total fungi | 0.244 | 0.017 | 0.074 | 0.11 | <0.001 | 0.31 | <0.001 |
| Total ciliate protozoa | 4.24 | 0.90 | 1.95 | 1.26 | <0.001 | 0.21 | <0.001 |
|  | Relative to d 0 of control5 | | |  |  |  |  |
| Total fungi | 0.78 | 0.06 | 0.27 | 0.41 | <0.001 | 0.45 | <0.001 |
| Total ciliate protozoa | 0.58 | 0.12 | 0.30 | 0.31 | <0.001 | 0.54 | <0.001 |
| Total bacteria | 0.65 | 0.68 | 0.75 | 0.16 | 0.21 | 0.38 | 0.02 |

1Control = high fibre low oil diet; Induction = promotion of milk fat depression by feeding a low fibre high oil diet; Recovery = high fibre low oil diet following induction of milk fat depression.

2Preplanned contrasts tested the difference between control and induction (*I* = *P* < 0.01, I = *P* < 0.05, and i = *P* < 0.10) and between control and recovery [R = *P* < 0.01, *R* = *P* < 0.05, and r = *P* < 0.10) when the treatment by time interaction was not significant (*P* > 0.15).

3Trt = Treatment effect; Time = effect of time; and Trt x time = Treatment by time interaction.

4Relative abundance (% of target gene relative to total bacterial 16S rDNA).

5Data standardized relative to d 0 of control (1.0).

**Supplemental Table 3.** Associations between milk fat, milk fatty acids and ruminal microbes

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | |  | |  | |  | |  | | Estimates1 | | | |  | | *P-*value2 | | | |
| Response/Predictor3 | | | R2 | | Adjusted R2 | | RMSE | |  | | Int | | L | Q |  | | Int | | L | Q |
| *Trans*-11 C18:1, % of 18C FA | | |  | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | Ciliate protozoa | | 0.08 | | 0.06 | | 1.13 | |  | | 2.27 | | 0.154 | -0.0251 |  | | <0.001 | | 0.09 | - |
|  | Anaerobic fungi | | 0.02 | | 0.00 | | 1.17 | |  | | 2.35 | | 1.274 | - |  | | <0.001 | | 0.13 | - |
|  | *Streptococcus bovis* | | 0.07 | | 0.05 | | 1.15 | |  | | 2.32 | | 23.26 | -153 |  | | <0.001 | | <0.01 | 0.02 |
|  | *Prevotella bryantii* | | 0.05 | | 0.04 | | 4.53 | |  | | 2.50 | | 0.673 | - |  | | <0.001 | | 0.58 | - |
|  | *Megasphaera eldesnii* | | 0.06 | | 0.05 | | 1.15 | |  | | 2.66 | | -9.82 | 8.10 |  | | <0.001 | | <0.01 | 0.01 |
|  | *Selenomonas ruminantium* | | 0.06 | | 0.05 | | 1.16 | |  | | 2.84 | | -1.62 | - |  | | <0.001 | | 0.11 | - |
|  | *Ruminococcus albus* | | 0.05 | | 0.04 | | 1.16 | |  | | 2.65 | | -0.637 | - |  | | <0.001 | | 0.66 | - |
|  | *Fibrobacter succinogenes* | | 0.04 | | 0.03 | | 1.17 | |  | | 2.45 | | 0.172 | - |  | | <0.001 | | 0.32 | - |
|  | *Prevotella ruminicola* | | 0.05 | | 0.04 | | 1.16 | |  | | 2.47 | | 0.118 | - |  | | <0.001 | | 0.67 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.13 | | 0.11 | | 1.12 | |  | | 2.00 | | 0.892 | -1.13 |  | | <0.001 | | <0.001 | 0.04 |
|  | *Butytivibrio hungatei* | | 0.03 | | 0.02 | | 1.17 | |  | | 2.21 | | 7.700 | - |  | | <0.001 | | 0.06 | - |
| Ciliate protozoa, % of total bacteria | | |  | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | Anaerobic fungi | | 0.57 | | 0.56 | | 2.08 | |  | | 1.36 | | 14.3 | -19.1 |  | | 0.04 | | <0.001 | 0.02 |
|  | *Streptococcus bovis* | | 0.24 | | 0.24 | | 2.74 | |  | | 3.91 | | -32.62 | - |  | | <0.01 | | <0.01 | - |
|  | *Prevotella bryantii* | | 0.18 | | 0.17 | | 2.84 | |  | | 3.56 | | -1.162 | - |  | | <0.01 | | 0.65 | - |
|  | *Megasphaera eldesnii* | | 0.19 | | 0.18 | | 2.82 | |  | | 5.88 | | -15.6 | 12.0 |  | | 0.01 | | 0.12 | - |
|  | *Selenomonas ruminantium* | | 0.33 | | 0.32 | | 2.58 | |  | | 5.23 | | -10.83 | - |  | | <0.001 | | <0.001 | - |
|  | *Ruminococcus albus* | | 0.27 | | 0.25 | | 2.70 | |  | | 2.35 | | 12.6 | -76.5 |  | | 0.01 | | <0.01 | <0.001 |
|  | *Fibrobacter succinogenes* | | 0.59 | | 0.58 | | 2.02 | |  | | 1.37 | | 3.54 | -1.012 |  | | <0.01 | | <0.001 | <0.001 |
|  | *Prevotella ruminicola* | | 0.25 | | 0.24 | | 2.73 | |  | | 1.35 | | 2.65 | -1.721 |  | | 0.17 | | 0.01 | <0.01 |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.44 | | 0.43 | | 2.36 | |  | | 0.166 | | 3.86 | -0.0412 |  | | 0.82 | | <0.001 | <0.001 |
|  | *Butytivibrio hungatei* | | 0.28 | | 0.26 | | 2.70 | |  | | 3.38 | | 14.34 | -730 |  | | <0.01 | | 0.35 | <0.01 |
| Anaerobic fungi, % of total bacteria | | |  | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Streptococcus bovis* | | 0.34 | | 0.33 | | 0.12 | |  | | 0.243 | | -6.04 | 31.6 |  | | <0.001 | | <0.001 | <0.001 |
|  | *Prevotella bryantii* | | 0.04 | | 0.03 | | 0.14 | |  | | 0.191 | | -0.162 | - |  | | <0.001 | | 0.19 | - |
|  | *Megasphaera eldesnii* | | 0.04 | | 0.03 | | 0.14 | |  | | 0.180 | | -0.302 | - |  | | 0.03 | | 0.01 | - |
|  | *Selenomonas ruminantium* | | 0.21 | | 0.20 | | 0.13 | |  | | 0.266 | | -0.554 | - |  | | <0.001 | | <0.001 | <0.001 |
|  | *Ruminococcus albus* | | 0.11 | | 0.10 | | 0.14 | |  | | 0.139 | | 0.456 | -3.49 |  | | <0.01 | | 0.05 | <0.01 |
|  | *Fibrobacter succinogenes* | | 0.76 | | 0.75 | | 0.07 | |  | | 0.078 | | 0.207 | -0.121 |  | | <0.01 | | <0.001 | <0.001 |
|  | *Prevotella ruminicola* | | 0.12 | | 0.10 | | 0.14 | |  | | 0.092 | | 0.107 | -0.0787 |  | | 0.04 | | 0.03 | <0.01 |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.39 | | 0.38 | | 0.11 | |  | | 0.0056 | | 0.199 | -0.00213 |  | | 0.83 | | <0.001 | <0.001 |
|  | *Butytivibrio hungatei* | | 0.18 | | 0.16 | | 0.13 | |  | | 0.15 | | 1.29 | -45.4 |  | | <0.01 | | 0.06 | <0.001 |
| *Streptococcus bovis,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Prevotella bryantii* | | 0.20 | | 0.19 | | 0.02 | |  | | 0.015 | | -0.0070 | - |  | | 0.07 | | 0.72 | - |
|  | *Megasphaera eldesnii* | | 0.21 | | 0.20 | | 0.02 | |  | | 0.014 | | 0.0081 | - |  | | 0.10 | | 0.68 | - |
|  | *Selenomonas ruminantium* | | 0.23 | | 0.22 | | 0.02 | |  | | 0.0092 | | 0.0315 | - |  | | 0.23 | | 0.10 | - |
|  | *Ruminococcus albus* | | 0.00 | | -0.01 | | 0.02 | |  | | 0.012 | | 0.0156 | - |  | | <0.01 | | 0.58 | - |
|  | *Fibrobacter succinogenes* | | 0.43 | | 0.42 | | 0.02 | |  | | 0.0253 | | -0.0195 | 0.00678 |  | | 0.01 | | <0.001 | 0.02 |
|  | *Prevotella ruminicola* | | 0.25 | | 0.24 | | - | |  | | 0.027 | | -0.015 | - |  | | <0.01 | | 0.01 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.24 | | 0.23 | | 0.02 | |  | | 0.022 | | -0.0089 | - |  | | 0.02 | | 0.08 | - |
|  | *Butytivibrio hungatei* | | 0.21 | | 0.21 | | 0.02 | |  | | 0.0036 | | 0.232 | - |  | | 0.56 | | 0.01 | - |
| *Prevotella bryantii,* % of total bacteria | | |  | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Megasphaera eldesnii* | | 0.23 | | 0.22 | | 0.11 | |  | | 0.103 | | -0.028 | - |  | | 0.03 | | 0.76 | - |
|  | *Selenomonas ruminantium* | | 0.23 | | 0.22 | | 0.02 | |  | | 0.051 | | 0.311 | - |  | | 0.12 | | <0.001 | - |
|  | *Ruminococcus albus* | | 0.15 | | 0.14 | | 0.11 | |  | | 0.132 | | -0.239 | - |  | | <0.001 | | 0.08 | - |
|  | *Fibrobacter succinogenes* | | 0.24 | | 0.23 | | 0.11 | |  | | 0.117 | | -0.021 | - |  | | 0.02 | | 0.19 | - |
|  | *Prevotella ruminicola* | | 0.25 | | 0.25 | | 0.10 | |  | | 0.137 | | -0.039 | - |  | | 0.01 | | 0.12 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.24 | | 0.23 | | 0.11 | |  | | 0.120 | | -0.046 | 0.120 |  | | 0.01 | | 0.07 | 0.03 |
|  | *Butytivibrio hungatei* | | 0.22 | | 0.21 | | 0.11 | |  | | 0.128 | | -0.554 | - |  | | 0.01 | | 0.18 | - |
| *Megasphaera eldesnii,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Selenomonas ruminantium* | | 0.15 | | 0.14 | | 0.11 | |  | | -0.019 | | 0.228 | - |  | | 0.40 | | 0.02 | - |
|  | *Ruminococcus albus* | | 0.12 | | 0.12 | | 0.11 | |  | | 0.053 | | -0.280 | - |  | | 0.02 | | 0.04 | - |
|  | *Fibrobacter succinogenes* | | 0.07 | | 0.06 | | 0.62 | |  | | 0.755 | | -5.86 | 4.47 |  | | 0.03 | | <0.01 | 0.01 |
|  | *Prevotella ruminicola* | | 0.12 | | 0.11 | | 0.11 | |  | | 0.024 | | -0.0061 | - |  | | 0.42 | | 0.82 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.15 | | 0.14 | | 0.11 | |  | | 0.058 | | -0.076 | 0.140 |  | | 0.04 | | <0.01 | 0.01 |
|  | *Butytivibrio hungatei* | | 0.11 | | 0.10 | | 0.11 | |  | | 0.047 | | -0.593 | - |  | | 0.08 | | 0.16 | - |
| *Selenomonas ruminantium,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Ruminococcus albus* | | 0.03 | | 0.02 | | 0.11 | |  | | 0.193 | | -0.219 | - |  | | <0.001 | | 0.08 | - |
|  | *Fibrobacter succinogenes* | | 0.21 | | 0.20 | | 0.57 | |  | | 1.40 | | -4.54 | 4.99 |  | | <0.001 | | <0.001 | <0.01 |
|  | *Prevotella ruminicola* | | 0.07 | | 0.06 | | 0.11 | |  | | 0.210 | | -0.0501 | - |  | | <0.001 | | 0.05 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.43 | | 0.42 | | 0.08 | |  | | 0.238 | | -0.137 | 0.244 |  | | <0.001 | | <0.001 | <0.001 |
|  | *Butytivibrio hungatei* | | 0.01 | | 0.003 | | 0.11 | |  | | 0.184 | | -0.404 | - |  | | <0.001 | | 0.25 | - |
| *Ruminococcus albus,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Fibrobacter succinogenes* | | 0.08 | | 0.06 | | 0.62 | |  | | 0.585 | | 1.60 | -13.30 |  | | <0.001 | | 0.13 | <0.01 |
|  | *Prevotella ruminicola* | | 0.06 | | 0.05 | | 0.08 | |  | | 0.114 | | 0.0102 | - |  | | <0.001 | | 0.59 | - |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.09 | | 0.08 | | 0.08 | |  | | 0.111 | | 0.038 | -0.107 |  | | <0.001 | | 0.02 | <0.01 |
|  | *Butytivibrio hungatei* | | 0.30 | | 0.30 | | 0.07 | |  | | 0.059 | | 1.36 | - |  | | <0.01 | | <0.001 | - |
| *Fibrobacter succinogenes,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Prevotella ruminicola* | | 0.14 | | 0.13 | | 0.60 | |  | | 0.124 | | 0.715 | -0.374 |  | | 0.51 | | <0.001 | <0.01 |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.24 | | 0.24 | | 0.55 | |  | | 0.039 | | 0.779 | - |  | | 0.73 | | <0.001 | - |
|  | *Butytivibrio hungatei* | | 0.17 | | 0.15 | | 0.59 | |  | | 0.641 | | 4.78 | -197 |  | | 0.01 | | 0.11 | <0.001 |
| *Prevotella ruminicola,* % of total bacteria | | | | |  | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *B. fibrisolvens/Pseudobutyrivibrio* | | 0.14 | | 0.12 | | 0.39 | |  | | 0.917 | | 0.0616 | -0.414 |  | | <0.001 | | 0.50 | 0.04 |
|  | *Butytivibrio hungatei* | | 0.09 | | 0.09 | | 0.39 | |  | | 0.835 | | 1.195 | - |  | | <0.001 | | 0.43 | - |
| *B. fibrisolvens/Pseudobutyrivibrio,* % of total bacteria | | | | | | |  | |  | |  | |  |  |  | |  | |  |  |
|  | *Butytivibrio hungatei* | | 0.25 | | 0.24 | | 0.38 | |  | | 0.562 | | 8.721 | -136 |  | | <0.01 | | <0.001 | <0.001 |

1Int = intercept; L = linear coefficient; Q = quadratic coefficient.

2Significant of the intercept (Int), linear (L), and quadratic (Q) terms. The quadratic effect was removed from the model when *P* > 0.05 only if model fit improved based on the AICc.

3Units of the predictors are % of total bacteria.



**Supplemental Figure 1**. Time course of abundance of total fungi (Panels A) and ciliate protozoa (Panels B) relative to total bacteria in rumen digesta of cows a high fibre, low oil diet (control), a low fibre, high oil diet resulting in milk fat depression (induction), or a high fibre, low oil diet following induction that resolved milk fat depression (recovery). Abundance is shown as the percent of total bacterial 16S rDNA. Preplanned contrasts tested the difference between control and induction (\*\*\* *P* < 0.01 and *\*\* P* < 0.05) and between control and recovery (††† *P* < 0.01). The largest SEM is reported.