**SUPPLEMENTARY TABLES AND FIGURES**

**Table S1.** Age, body weight, daily weight gain, and frequency of medications in the calves during the suckling period (Experiment 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | | Control | Trehalose | *P*-value 1 |
| Age, days | |  |  |  |
|  | Beginning of trial (Day 1) | 11.9 ± 2.0 | 11.4 ± 2.0 | 0.60 |
|  | Three weeks after trehalose feeding (Day 22) | 32.9 ± 2.0 | 32.4 ± 2.0 | 0.60 |
|  | One day before weaning (Day 55) 2 | 65.3 ± 9.0 | 64.3 ± 4.5 | 0.77 |
| Body weight, kg | |  |  |  |
|  | Day 1 | 45.2 ± 5.1 | 45.3 ± 4.8 | 0.97 |
|  | Day 55 | 83.1 ± 10.5 | 85.5 ± 5.6 | 0.56 |
|  | Average daily weight gain, kg/day/animal | 0.58 ± 0.10 | 0.63 ± 0.09 | 0.26 |
| Number of days for medication, days/animal 3 | | 2.3 ± 3.6 | 2.7 ± 1.8 | 0.77 |

Values are shown as mean ± SD.

1 *P*-values were calculated based on Welch’s t-test.

2 Calves were weaned when more than 1.5 kg of calf starter consumption was observed for 3 consecutive days.

3 Not only diarrhea-related causes but also other causes, including respiratory diseases, were counted. The necessity of medication was carefully judged by the experienced professional farmers.

**Table S2**. Bacterial primers and their target bacterial group used for the determination of population size by real-time PCR

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Target | Primer sequences (5′-3′) | Annealing | Extension | Product | Reference |
| temperature (℃) | time (s) | size (bp) |
| Total bacteria | [F] CCTACGGGAGGCAGCAG | 60 | 1 | 194 | Muyzer et al., 1993 |
| [R] ATTACCGCGGCTGCTGG |
| Ruminococcaceae | [F] GCACAAGCAGTGGAKT | 50 | 1 | 239 | Matsuki et al., 2004 modified |
| [R] CTTCCTCCGTTTTGTCAA |
| *Bacteroides-Prevotella-Porphyromonas* | [F] GGTGTCGGCTTAAGTGCCAT | 68 | 1 | 140 | Rinttila et al., 2004 |
| [R] CGGA(C/T)GTAAGGGCCGTGC |
| *Prevotella* spp. | [F] CACRGTAAACGATGGATGCC | 55 | 2 | 534 | Matuki et al., 2002 |
| [R] GGTCGGGTTGCAGACC |
| *Faecalibacterium prausnitzii* | [F] GGAGGAAGAAGGTCTTCGG | 60 | 1 | 248 | Wamg et al., 1996, Ramirez et al., 2009 |
| [R] AATTCCGCCTACCTCTGCACT |
| *Lactobacillus* spp. | [F] AGCAGTAGGGAATCTTCCA | 58 | 2 | 341 | Walter et al., 1996, Heilig et al., 2002 |
| [R] CACCGCTACACATGGAG |
| *Clostridium coccoides - Eubacterium rectale* group | [F] CGGTACCTGACTAAGAAGC | 55 | 1 | 429 | Rinttila et al., 2004 |
| [R] AGTTT(C/T)ATTCTTGCGAACG |
| *Bifidobacterium* spp. | [F] GGGTGGTAATGCCGGATG | 62 | 2 | 523 | Kok et al., 1996 |
| [R] CCACCGTTACACCGGGAA |
| *Escherichia coli* | [F] GTTAATACCTTTGCTCATTGA | 60 | 1 | 340 | Malinen et al., 2003 |
| [R] ACCAGGGTATCTAATCCTGTT |
| *Akkermansia muciniphila* | [F] CAGCACGTGAAGGTGGGGAC | 60 | 1 | 327 | Png et al., 2010 |
| [R] CCTTGCGGTTGGCTTCAGAT |
| *Clostridium perfringens* | [F] AAAGATGGCATCATCATTCAAC | 55 | 1 | 279 | Wang et al., 1994 |
| [R] TACCGTCATTATCTTCCCCAAA |
| *Eubacterium hallii* | [F] GCGTAGGTGGCAGTGCAA | 60 | 1 | 278 | Hold et al., 2003, Ramirez et al., 2009 |
| [R] GCACCGRAGCCTATACGG |
| *Streptococcus bovis* | [F] CTAATACCGCATAACAGCAT | 57 | 3 | 869 | Tajima et al., 2001 |
| [R] AGAAACTTCCTATCTCTAGG |
| *Megasphaera elsdenii* | [F] GACCGAAACTGCGATGCTAGA | 58 | 1 | 130 | Ouwerkerk et al., 2002 |
| [R] CGCCTCAGCGTCAGTTGTC |

**Table S3.** Results of statistical test by using repeated measure model in fecal fermentation parameters, alpha diversity and bacterial abundances in preweaned calves

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Item 2 | | | Day 22 | |  | Day 55 | |  | *P-*value 1 | | |
| Control | Trehalose |  | Control | Trehalose |  | Day | Treatment | Day×Treatment |
| Fecal fermentation parameters | | |  |  |  |  |  |  |  |  |  |
|  | pH | | 7.15 ± 0.49 | 7.23 ± 0.48 |  | 7.66 ± 0.30 | 7.55 ± 0.43 |  | 0.006 | 0.584 | 0.525 |
|  | Total VFA, μmol/g feces | | 48.9 ± 11.9 | 56.0 ± 17.9 |  | 76.3 ± 15.9 | 84.5 ± 34.2 |  | < 0.001 | 0.727 | 0.943 |
|  |  | Acetate, mol/100 mol | 55.5 ± 5.0 | 52.9 ± 5.3 |  | 60.0 ± 5.4 | 58.4 ± 2.7 |  | 0.001 | 0.382 | 0.732 |
|  |  | Propionate, mol/100 mol | 27.6 ± 3.0 | 28.0 ± 3.5 |  | 25.9 ± 2.4 | 27.8 ± 3.5 |  | 0.303 | 0.800 | 0.418 |
|  |  | Butyrate, mol/100 mol | 11.9 ± 2.7 | 14.5 ± 3.3 |  | 8.8 ± 1.6 | 9.4 ± 1.8 |  | < 0.001 | 0.054 | 0.191 |
|  |  | Isobutyrate, mol/100 mol | 1.2 ± 0.7 | 1.6 ± 1.2 |  | 1.4 ± 0.8 | 1.9 ± 1.0 |  | 0.204 | 0.687 | 0.679 |
|  |  | Isovalerate, mol/100 mol | 1.5 ± 0.9 | 1.9 ± 1.7 |  | 1.5 ± 1.0 | 1.4 ± 1.1 |  | 0.297 | 0.412 | 0.319 |
|  |  | Valerate, mol/100 mol | 2.3 ± 1.8 | 1.2 ± 1.2 |  | 2.3 ± 1.5 | 1.2 ± 0.6 |  | 0.962 | 0.196 | 0.972 |
|  | Ammonia, μgN/g feces | | 0.27 ± 0.12 | 0.30 ± 0.34 |  | 0.21 ± 0.12 | 0.23 ± 0.14 |  | 0.362 | 0.854 | 0.950 |
|  | Total lactate, μmol/g feces | | 5.51 ± 3.14 | 6.31 ± 7.71 |  | 14.59 ± 5.67 | 13.74 ± 8.94 |  | 0.001 | 0.742 | 0.717 |
|  | Indole, μg/g feces | | 12.67 ± 8.52 | 14.02 ± 18.54 |  | 6.62 ± 3.33 | 7.77 ± 3.32 |  | 0.087 | 0.866 | 0.977 |
|  | Skatole, μg/g feces | | 104.8 ± 46.4 | 119.5 ± 101.3 |  | 77.4 ± 30.5 | 87.9 ± 30.4 |  | 0.123 | 0.718 | 0.911 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Alpha diversity in fecal microbiota | | |  |  |  |  |  |  |  |  |  |
|  | Chao1 | | 671.1 ± 172.5 | 623.0 ± 113.1 |  | 777.6 ± 107.1 | 778.4 ± 97.0 |  | 0.004 | 0.435 | 0.541 |
|  | ACE | | 644.5 ± 162.3 | 603.6 ± 90.2 |  | 757.0 ± 100.3 | 771.9 ± 83.2 |  | 0.002 | 0.422 | 0.468 |
|  | Shannon | | 5.42 ± 0.68 | 5.41 ± 0.49 |  | 6.59 ± 0.31 | 6.30 ± 0.45 |  | < 0.001 | 0.666 | 0.401 |
|  | Simpson | | 0.93 ± 0.03 | 0.94 ±0.02 |  | 0.97 ± 0.01 | 0.95 ± 0.01 |  | < 0.001 | 0.172 | 0.139 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Relative abundances of genus-level bacterial taxa in fecal microbiota 3 | | | | |  |  |  |  |  |  |  |
|  | *Prevotella* | | 12.080 ± 9.203 | 15.852 ± 10.460 |  | 17.496 ± 8.234 | 15.570 ± 11.636 |  | 0.418 | 0.355 | 0.370 |
|  | *Bacteroides* | | 10.190 ± 9.315 | 7.523 ± 7.211 |  | 8.742 ± 4.697 | 5.970 ± 4.238 |  | 0.463 | 0.626 | 0.980 |
|  | Unclassified Clostridiales | | 9.350 ± 8.898 | 6.670 ± 4.433 |  | 7.783 ± 1.837 | 8.724 ± 4.364 |  | 0.896 | 0.281 | 0.330 |
|  | *Faecalibacterium* | | 7.580 ± 4.987 | 9.219 ± 9.494 |  | 3.214 ± 1.561 | 4.050 ± 2.037 |  | 0.018 | 0.644 | 0.829 |
|  | Unclassified Ruminococcaceae | | 7.540 ± 4.376 | 7.274 ± 5.329 |  | 16.493 ± 4.128 | 13.288 ± 5.603 |  | < 0.001 | 0.675 | 0.355 |
|  | Unclassified S24-7 | | 6.960 ± 8.632 | 2.658 ± 3.798 |  | 10.268 ± 3.309 | 15.511 ± 5.709 |  | < 0.001 | 0.035 | 0.020 |
|  | [Prevotella] | | 5.440 ± 5.901 | 4.354 ± 4.231 |  | 2.894 ± 2.758 | 5.930 ± 2.765 |  | 0.714 | 0.265 | 0.131 |
|  | Unclassified Enterobacteriaceae | | 5.240 ± 13.322 | 0.691 ± 0.951 |  | 0.099 ± 0.128 | 0.104 ± 0.159 |  | 0.206 | 0.188 | 0.313 |
|  | *Phascolarctobacterium* | | 3.640 ± 3.024 | 3.808 ± 3.102 |  | 2.814 ± 1.634 | 3.621 ± 2.318 |  | 0.383 | 0.881 | 0.580 |
|  | *Ruminococcus* | | 3.630 ± 5.781 | 1.164 ± 1.066 |  | 3.876 ± 3.314 | 3.278 ± 1.429 |  | 0.294 | 0.198 | 0.402 |
|  | *Blautia* | | 2.550 ± 1.390 | 3.862 ± 2.464 |  | 1.426 ± 0.636 | 1.037 ± 0.704 |  | 0.001 | 0.060 | 0.097 |
|  | Unclassified Lachnospiraceae | | 2.480 ± 1.268 | 2.627 ± 1.088 |  | 2.877 ± 0.790 | 2.633 ± 1.346 |  | 0.589 | 0.668 | 0.600 |
|  | *Oscillospira* | | 2.280 ± 1.068 | 2.660 ± 0.804 |  | 3.215 ± 0.967 | 2.564 ± 1.688 |  | 0.293 | 0.291 | 0.198 |
|  | Unclassified Bacteroidales | | 2.120 ± 1.746 | 4.150 ± 8.155 |  | 4.269 ± 4.728 | 3.296 ± 2.953 |  | 0.640 | 0.287 | 0.286 |
|  | *Parabacteroides* | | 1.790 ± 1.217 | 2.000 ± 1.637 |  | 2.604 ± 1.190 | 2.131 ± 1.235 |  | 0.294 | 0.558 | 0.446 |
|  | *Clostridium* | | 1.570 ± 1.347 | 0.448 ± 0.390 |  | 2.418 ± 1.538 | 1.808 ± 1.178 |  | 0.009 | 0.155 | 0.521 |
|  | [Ruminococcus] | | 1.320 ± 2.351 | 0.800 ± 0.761 |  | 0.024 ± 0.029 | 0.032 ± 0.027 |  | 0.017 | 0.412 | 0.528 |
|  | *Butyricicoccus* | | 1.220 ± 2.393 | 0.241 ± 0.159 |  | 0.064 ± 0.055 | 0.063 ± 0.104 |  | 0.105 | 0.118 | 0.231 |
|  | *Megasphaera* | | 1.100 ± 1.982 | 1.038 ± 1.346 |  | 0.056 ± 0.074 | 0.091 ± 0.153 |  | 0.023 | 0.900 | 0.903 |
|  | *Succinivibrio* | | 1.090 ± 1.409 | 1.105 ± 1.223 |  | 0.461 ± 0.730 | 1.236 ± 2.045 |  | 0.529 | 0.643 | 0.342 |
|  | *Dorea* | | 1.000 ± 0.983 | 1.570 ± 2.344 |  | 0.762 ± 0.532 | 0.734 ± 0.786 |  | 0.099 | 0.292 | 0.341 |
|  | *Sutterella* | | 0.990 ± 0.956 | 1.032 ± 0.919 |  | 1.038 ± 0.478 | 0.821 ± 0.343 |  | 0.737 | 0.729 | 0.596 |
|  | *Collinsella* | | 0.710 ± 0.816 | 1.190 ± 1.295 |  | 0.414 ± 0.263 | 0.423 ± 0.531 |  | 0.012 | 0.163 | 0.230 |
|  | Unclassified Coriobacteriaceae | | 0.660 ± 0.653 | 0.876 ± 0.603 |  | 0.773 ± 0.349 | 0.435 ± 0.209 |  | 0.317 | 0.168 | 0.099 |
|  | Unclassified Clostridiaceae | | 0.640 ± 1.791 | 0.013 ± 0.032 |  | 0.189 ± 0.171 | 0.287 ± 0.247 |  | 0.769 | 0.156 | 0.238 |
|  | *Anaerovibrio* | | 0.580 ± 0.893 | 0.680 ± 1.022 |  | 0.616 ± 1.327 | 0.330 ± 0.505 |  | 0.434 | 0.567 | 0.336 |
|  | *Lachnobacterium* | | 0.570 ± 0.990 | 0.788 ± 2.023 |  | 0.027 ± 0.059 | 0.224 ± 0.579 |  | 0.161 | 0.818 | 0.981 |
|  | Unclassified Erysipelotrichaceae | | 0.490 ± 0.323 | 0.604 ± 0.366 |  | 0.240 ± 0.256 | 0.163 ± 0.155 |  | 0.001 | 0.317 | 0.317 |
|  | *Bifidobacterium* | | 0.440 ± 0.690 | 0.838 ± 2.136 |  | 0.003 ± 0.004 | 0.003 ± 0.004 |  | 0.098 | 0.488 | 0.597 |
|  | *Dialister* | | 0.380 ± 0.693 | 3.680 ± 4.577 |  | 0.036 ± 0.038 | 0.182 ± 0.214 |  | 0.018 | 0.009 | 0.048 |
|  | *Megamonas* | | 0.350 ± 0.477 | 0.320 ± 0.362 |  | 0.242 ± 0.257 | 0.473 ± 0.658 |  | 0.866 | 0.558 | 0.323 |
|  | *Coprococcus* | | 0.350 ± 0.284 | 0.188 ± 0.186 |  | 0.506 ± 0.305 | 0.850 ± 0.493 |  | 0.001 | 0.089 | 0.030 |
|  | CF231 | | 0.320 ± 0.428 | 0.246 ± 0.402 |  | 0.810 ± 0.630 | 0.389 ± 0.429 |  | 0.050 | 0.695 | 0.266 |
|  | *Mitsuokella* | | 0.290 ± 0.645 | 2.659 ± 5.819 |  | 0.008 ± 0.014 | 0.045 ± 0.088 |  | 0.147 | 0.121 | 0.239 |
|  | *Odoribacter* | | 0.180 ± 0.332 | 0.180 ± 0.295 |  | 0.152 ± 0.072 | 0.314 ± 0.229 |  | 0.532 | 0.619 | 0.343 |
|  | p-75-a5 | | 0.170 ± 0.203 | 0.401 ± 1.038 |  | 0.205 ± 0.241 | 0.510 ± 0.768 |  | 0.640 | 0.677 | 0.825 |
|  | [Eubacterium] | | 0.130 ± 0.119 | 0.352 ± 0.332 |  | 0.180 ± 0.162 | 0.310 ± 0.524 |  | 0.963 | 0.289 | 0.651 |
|  | Unclassified YS2 | | 0.100 ± 0.135 | 0.093 ± 0.104 |  | 0.119 ± 0.158 | 0.181 ± 0.164 |  | 0.275 | 0.625 | 0.447 |
|  | *Lachnospira* | | 0.070 ± 0.134 | 0.257 ± 0.355 |  | 0.025 ± 0.027 | 0.110 ± 0.096 |  | 0.147 | 0.137 | 0.446 |
|  | Unclassified Rikenellaceae | | 0.070 ± 0.144 | 0.028 ± 0.035 |  | 0.475 ± 0.733 | 0.207 ± 0.162 |  | 0.033 | 0.744 | 0.386 |
|  | Unclassified RF39 | | 0.030 ± 0.041 | 0.018 ± 0.021 |  | 0.082 ± 0.095 | 0.091 ± 0.049 |  | 0.003 | 0.596 | 0.589 |
|  | *Desulfovibrio* | | 0.010 ± 0.013 | 0.025 ± 0.035 |  | 0.145 ± 0.110 | 0.252 ± 0.367 |  | 0.008 | 0.767 | 0.471 |
|  | Unclassified Christensenellaceae | | 0.010 ± 0.021 | 0.000 ± 0.000 |  | 0.222 ± 0.243 | 0.081 ± 0.119 |  | 0.004 | 0.488 | 0.151 |
|  | Other | | 2.280 ± 2.838 | 5.819 ± 9.902 |  | 1.640 ± 0.555 | 1.652 ± 0.649 |  | 0.176 | 0.187 | 0.315 |

Values are shown as mean ± SD.

1 *P*-values of fixed effects were calculated by repeated measures model.

2 Data on fecal score were not included in Table S3 due to the difference in the time points (i.e., monitoring for every day through the suckling period). Statistical significance was observed in Day (*P* < 0.001) and Day × Treatment (*P* = 0.001), but not in Treatment (*P* = 0.218).

3 Values mean relative abundance (% of total reads). Name of taxa with square brackets indicates putative assignment.

**Table S4.** Alpha diversity indices of fecal microbiota in preweaned calves at Day 22 and Day 55 (Experiment 2)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Indices | Day 22 | | |  | Day 55 | | |
|  | Control | Trehalose | *P-*value 1 |  | Control | Trehalose | *P-*value 1 |
| Chao1 | 671.1 ± 172.5 | 623.0 ± 113.1 | 0.495 |  | 777.6 ± 107.1 | 778.4 ± 97.0 | 0.987 |
| ACE | 644.5 ± 162.3 | 603.6 ± 90.2 | 0.520 |  | 757.0 ± 100.3 | 771.9 ± 83.2 | 0.735 |
| Shannon | 5.42 ± 0.68 | 5.41 ± 0.49 | 0.980 |  | 6.59 ± 0.31 | 6.30 ± 0.45 | 0.136 |
| Simpson | 0.93 ± 0.03 | 0.94 ±0.02 | 0.433 |  | 0.97 ± 0.01 | 0.95 ± 0.01 | 0.104 |

Alpha diversity indices were calculated using the QIIME.

Values are shown as mean ± SD.

1 Each parameter was compared between the control and trehalose groups at respective days. *P*-values were calculated by Welch’s t-test.

**Table S5.** Relative abundances of bacterial taxa showing 0.1% or higher relative abundance in at least ≥5 individuals within any group at Day 22 or Day 55

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Taxa | Day 22 | | |  | Day 55 | | |
| Control | Trehalose | *P-*value 1 |  | Control | Trehalose | *P-*value 1 |
| *Prevotella* | 12.080 ± 9.203 | 15.852 ± 10.460 | 0.427 |  | 17.496 ± 8.234 | 15.570 ± 11.636 | 0.690 |
| *Bacteroides* | 10.190 ± 9.315 | 7.523 ± 7.211 | 0.506 |  | 8.742 ± 4.697 | 5.970 ± 4.238 | 0.205 |
| Unclassified Clostridiales | 9.350 ± 8.898 | 6.670 ± 4.433 | 0.432 |  | 7.783 ± 1.837 | 8.724 ± 4.364 | 0.562 |
| *Faecalibacterium* | 7.580 ± 4.987 | 9.219 ± 9.494 | 0.654 |  | 3.214 ± 1.561 | 4.050 ± 2.037 | 0.343 |
| Unclassified Ruminococcaceae | 7.540 ± 4.376 | 7.274 ± 5.329 | 0.910 |  | 16.493 ± 4.128 | 13.288 ± 5.603 | 0.185 |
| Unclassified S24-7 | 6.960 ± 8.632 | 2.658 ± 3.798 | 0.196 |  | 10.268 ± 3.309 | 15.511 ± 5.709 | 0.031 |
| [Prevotella] | 5.440 ± 5.901 | 4.354 ± 4.231 | 0.659 |  | 2.894 ± 2.758 | 5.930 ± 2.765 | 0.031 |
| Unclassified Enterobacteriaceae | 5.240 ± 13.322 | 0.691 ± 0.951 | 0.333 |  | 0.099 ± 0.128 | 0.104 ± 0.159 | 0.939 |
| *Phascolarctobacterium* | 3.640 ± 3.024 | 3.808 ± 3.102 | 0.908 |  | 2.814 ± 1.634 | 3.621 ± 2.318 | 0.406 |
| *Ruminococcus* | 3.630 ± 5.781 | 1.164 ± 1.066 | 0.237 |  | 3.876 ± 3.314 | 3.278 ± 1.429 | 0.628 |
| *Blautia* | 2.550 ± 1.390 | 3.862 ± 2.464 | 0.186 |  | 1.426 ± 0.636 | 1.037 ± 0.704 | 0.235 |
| Unclassified Lachnospiraceae | 2.480 ± 1.268 | 2.627 ± 1.088 | 0.794 |  | 2.877 ± 0.790 | 2.633 ± 1.346 | 0.646 |
| *Oscillospira* | 2.280 ± 1.068 | 2.660 ± 0.804 | 0.404 |  | 3.215 ± 0.967 | 2.564 ± 1.688 | 0.332 |
| Unclassified Bacteroidales | 2.120 ± 1.746 | 4.150 ± 8.155 | 0.482 |  | 4.269 ± 4.728 | 3.296 ± 2.953 | 0.608 |
| *Parabacteroides* | 1.790 ± 1.217 | 2.000 ± 1.637 | 0.760 |  | 2.604 ± 1.190 | 2.131 ± 1.235 | 0.419 |
| *Clostridium* | 1.570 ± 1.347 | 0.448 ± 0.390 | 0.036 |  | 2.418 ± 1.538 | 1.808 ± 1.178 | 0.358 |
| [Ruminococcus] | 1.320 ± 2.351 | 0.800 ± 0.761 | 0.542 |  | 0.024 ± 0.029 | 0.032 ± 0.027 | 0.594 |
| *Butyricicoccus* | 1.220 ± 2.393 | 0.241 ± 0.159 | 0.253 |  | 0.064 ± 0.055 | 0.063 ± 0.104 | 0.986 |
| *Megasphaera* | 1.100 ± 1.982 | 1.038 ± 1.346 | 0.938 |  | 0.056 ± 0.074 | 0.091 ± 0.153 | 0.539 |
| *Succinivibrio* | 1.090 ± 1.409 | 1.105 ± 1.223 | 0.986 |  | 0.461 ± 0.730 | 1.236 ± 2.045 | 0.307 |
| *Dorea* | 1.000 ± 0.983 | 1.570 ± 2.344 | 0.511 |  | 0.762 ± 0.532 | 0.734 ± 0.786 | 0.931 |
| *Sutterella* | 0.990 ± 0.956 | 1.032 ± 0.919 | 0.926 |  | 1.038 ± 0.478 | 0.821 ± 0.343 | 0.284 |
| *Collinsella* | 0.710 ± 0.816 | 1.190 ± 1.295 | 0.362 |  | 0.414 ± 0.263 | 0.423 ± 0.531 | 0.964 |
| Unclassified Coriobacteriaceae | 0.660 ± 0.653 | 0.876 ± 0.603 | 0.480 |  | 0.773 ± 0.349 | 0.435 ± 0.209 | 0.025 |
| Unclassified Clostridiaceae | 0.640 ± 1.791 | 0.013 ± 0.032 | 0.320 |  | 0.189 ± 0.171 | 0.287 ± 0.247 | 0.343 |
| *Anaerovibrio* | 0.580 ± 0.893 | 0.680 ± 1.022 | 0.827 |  | 0.616 ± 1.327 | 0.330 ± 0.505 | 0.557 |
| *Lachnobacterium* | 0.570 ± 0.990 | 0.788 ± 2.023 | 0.779 |  | 0.027 ± 0.059 | 0.224 ± 0.579 | 0.336 |
| Unclassified Erysipelotrichaceae | 0.490 ± 0.323 | 0.604 ± 0.366 | 0.483 |  | 0.240 ± 0.256 | 0.163 ± 0.155 | 0.452 |
| *Bifidobacterium* | 0.440 ± 0.690 | 0.838 ± 2.136 | 0.605 |  | 0.003 ± 0.004 | 0.003 ± 0.004 | 0.987 |
| *Dialister* | 0.380 ± 0.693 | 3.680 ± 4.577 | 0.060 |  | 0.036 ± 0.038 | 0.182 ± 0.214 | 0.073 |
| *Megamonas* | 0.350 ± 0.477 | 0.320 ± 0.362 | 0.875 |  | 0.242 ± 0.257 | 0.473 ± 0.658 | 0.348 |
| *Coprococcus* | 0.350 ± 0.284 | 0.188 ± 0.186 | 0.175 |  | 0.506 ± 0.305 | 0.850 ± 0.493 | 0.095 |
| CF231 | 0.320 ± 0.428 | 0.246 ± 0.402 | 0.703 |  | 0.810 ± 0.630 | 0.389 ± 0.429 | 0.117 |
| *Mitsuokella* | 0.290 ± 0.645 | 2.659 ± 5.819 | 0.254 |  | 0.008 ± 0.014 | 0.045 ± 0.088 | 0.251 |
| *Odoribacter* | 0.180 ± 0.332 | 0.180 ± 0.295 | 0.996 |  | 0.152 ± 0.072 | 0.314 ± 0.229 | 0.068 |
| p-75-a5 | 0.170 ± 0.203 | 0.401 ± 1.038 | 0.519 |  | 0.205 ± 0.241 | 0.510 ± 0.768 | 0.280 |
| [Eubacterium] | 0.130 ± 0.119 | 0.352 ± 0.332 | 0.083 |  | 0.180 ± 0.162 | 0.310 ± 0.524 | 0.493 |
| Unclassified YS2 | 0.100 ± 0.135 | 0.093 ± 0.104 | 0.853 |  | 0.119 ± 0.158 | 0.181 ± 0.164 | 0.423 |
| *Lachnospira* | 0.070 ± 0.134 | 0.257 ± 0.355 | 0.171 |  | 0.025 ± 0.027 | 0.110 ± 0.096 | 0.027 |
| Unclassified Rikenellaceae | 0.070 ± 0.144 | 0.028 ± 0.035 | 0.401 |  | 0.475 ± 0.733 | 0.207 ± 0.162 | 0.311 |
| Unclassified RF39 | 0.030 ± 0.041 | 0.018 ± 0.021 | 0.441 |  | 0.082 ± 0.095 | 0.091 ± 0.049 | 0.806 |
| *Desulfovibrio* | 0.010 ± 0.013 | 0.025 ± 0.035 | 0.294 |  | 0.145 ± 0.110 | 0.252 ± 0.367 | 0.420 |
| Unclassified Christensenellaceae | 0.010 ± 0.021 | 0.000 ± 0.000 | 0.251 |  | 0.222 ± 0.243 | 0.081 ± 0.119 | 0.143 |
| Others | 2.280 ± 2.838 | 5.819 ± 9.902 | 0.326 |  | 1.640 ± 0.555 | 1.652 ± 0.649 | 0.967 |

Name of taxa with square brackets indicates putative assignment.

Values mean relative abundance (% of total reads) and are shown as mean ± SD.

1 Abundance of each bacterial taxa was compared between the control and trehalose groups at respective days. *P*-values were calculated by Welch’s t-test.

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**Figure Legends**

**Figure S1.** Principal coordinate analysis of microbial community structure in preweaned calves at Day 22 and Day 55 based on the Bray–Curtis dissimilarity matrices at the genus level (Experiment 2). Individual plots represent individual calves. Colors of plots indicate the experimental day; Day 22 (gray) and Day 55 (black). Shapes of plots indicate group; control group (circle) and trehalose group (triangle).