**Effects of feed withdrawal duration on animal behaviour, rumen microbiota and blood chemistry in feedlot cattle: implications for rumen acidosis**

A. Rabaza, G. Banchero, C. Cajarville, P. Zunino, A. Britos, J. L. Repetto and M. Fraga

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**Supplementary material**

**Supplementary Material S1** Results of quality control tests

For volatile fatty acids determination, the inter-assay CV was 10.02%. For ammonia nitrogen analyses the intra-assay CV and inter-assay CV were 11.2 and 9.11 %, respectively. The sensitivity of the glucose determination assay was 0.23 mg/dL. The intra-assay CVs for control 1 (91.2 mg/dL) and control 2 (258.0 mg/dL) were 3.59% and 3.36% respectively. For ammonia nitrogen quantification the sensitivity of the assay was 0.01mmol/L. The intra-assay CVs for control 1 (1.05 mmol/L) was 2.51%. In the case of the insulin determination, the sensitivity was 0.76 μUI/mL. The intra-assay CVs for control 1 (17.0 μUI/mL) and control 2 (78.6 μUI/mL) were 5.38% and 3.96% respectively. For haptoglobin serum quantification, the sensitivity of the assay was 0.005 mg/mL. The intra-assay CVs for control 1 (0.43 mg/mL) and control 2 (1.07 mg/mL) were 14.42% and 6.83% respectively. The inter-assay CVs for control 1 (0.42 mg/mL) and control 2 (1.06 mg/mL) were 12.79% and 9.41% respectively.

Treatment: *P* < 0.05

**Supplementary Figure S1** Pattern of ruminal pH of feedlot steers during the study. The feed withdrawal-feed reintroduction challenge was applied for 12 h in T12, 24 h in T24 and for 36 h in T36, whereas the control group had no feed withdrawal (C). Time 0 h represents the beginning of the withdrawal period. The means from 5 animals in each treatment are presented. The SEM is represented by bars.

**Supplementary Table S1** Mean, median, maximum, minimum and range of ruminal pH of feedlot steers during pre-feed withdrawal, feed withdrawal, reintroduction of feed and post-reintroduction of feed period (concentrate:forage = 85:15) (n = 20)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment1 | Phases2 | Mean3 | SEM | Median3 | Maximum | Minimum | Range | *P* – value4 |
| FW | T | (FW x T) |
| CON |  | 5.8 a | 0.09 | 5.7 | 6.4 | 5.2 | 1.25 | *\** | *\** |  |
|  |  |  |  |  |  |  |  |  |  |  |
| T12 | pre-FW | 5.5 a | 0.11 | 5.3 | 6.7 | 4.7 | 1.99 |  |  |  |
|  | FW | 6.6 b | 0.11 | 6.7 | 7.0 | 5.9 | 1.12 |  |  |  |
|  | FR | 5.1 b | 0.11 | 5.1 | 5.5 | 4.6 | 0.84 |  |  |  |
|  | post-FR | 5.8 a | 0.11 | 5.6 | 7.1 | 4.6 | 2.55 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| T24 | pre-FW | 6.0 a | 0.10 | 6.0 | 6.9 | 5.4 | 1.43 |  |  |  |
| FW | 6.9 b | 0.10 | 6.9 | 7.7 | 6.2 | 1.49 |  |  |  |
| FR | 5.3 b | 0.10 | 5.2 | 6.6 | 4.3 | 2.31 |  |  |  |
| post-FR | 5.9 a | 0.10 | 5.8 | 7.2 | 4.7 | 2.52 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| T36 | pre-FW | 5.9 a | 0.09 | 6.1 | 7.0 | 5.2 | 1.79 |  |  |  |
| FW | 7.0 b | 0.09 | 7.2 | 8.0 | 5.9 | 2.15 |  |  |  |
| FR | 4.8 b | 0.09 | 4.6 | 7.3 | 4.2 | 3.05 |  |  |  |
| post-FR | 5.7 a | 0.09 | 5.6 | 7.4 | 4.2 | 3.26 |   |   |   |

1 Treatment: CON = no feed withdrawal; T12 = 12 h of feed withdrawal; T24 = 24 h of feed withdrawal; T36 = 36 h of feed withdrawal.

2 Phases: pre-FW: pre-feed withdrawal; FW: feed withdrawal; FR: reintroduction of feed; post-FR: post- reintroduction of feed.

3 Data was co-variate adjusted with pre-feed withdrawal measurements, effect of co-variant = *P* < 0.05.

4 *P* – value; level of significance of the effect of Treatment (FW); Time (T) and Treatment x time (FW x T); \* *P* < 0.05.

a, b Within each phase the values with different superscripts differ significantly at *P* < 0.05.

**Supplementary Table S2** Relative abundance expressed as proportional data of bacterial genera in feedlot steers measured at feed reintroduction and 12 hours after feed reintroduction

|  |  |  |
| --- | --- | --- |
| Bacteria genera | Treatment1 | *Significance* |
| CON | T12 | T24 | T36 |
| FR | 12 h-FR | FR | 12 h-FR | FR | 12 h-FR | FR | 12 h-FR | FW | T |
| *[Eubacterium]* | 0.0004 | 0.0004 | 0.0003 | 0.0051 | 0.0002 | 0.0024 | 0.0001 | 0.0015 |  |  |
| *5-7N15* | 0 | 0 | 1.78E-06 | 0 | 7.94E-07 | 1.06E-05 | 2.83E-06 | 1.52E-05 |  |  |
| *Acholeplasma* | 3.96E-07 | 0 | 5.09E-06 | 0 | 0 | 0 | 0 | 9.88E-07 |  |  |
| *Acidaminococcus* | 0.0030 | 0.0010 | 0.0011 | 0.0009 | 0.0001 | 0.0003 | 6.79E-05 | 0.0009 |  |  |
| *Acinetobacter* | 9.39E-06 | 2.83E-05 | 2.89E-05 | 5.58E-05 | 3.65E-05 | 0.0002 | 0.0010 | 0.0002 |  |  |
| *Actinoalloteichus* | 1.85E-06 | 2.47E-06 | 0 | 3.96E-06 | 4.53E-06 | 1.04E-05 | 1.74E-06 | 1.19E-05 |  |  |
| *Actinobacillus* | 0 | 0 | 0 | 0 | 7.94E-07 | 1.47E-06 | 7.29E-06 | 2.87E-05 |  |  |
| *Actinomadura* | 0 | 0 | 0 | 0 | 7.94E-07 | 1.56E-06 | 0 | 0 |  |  |
| *Actinomycetospora* | 0 | 0 | 0 | 2.88E-06 | 8.58E-05 | 8.16E-07 | 0 | 9.88E-07 |  |  |
| *Adlercreutzia* | 2.13E-06 | 8.04E-06 | 3.30E-05 | 3.36E-05 | 0.0001 | 1.71E-05 | 0.0001 | 2.31E-05 |  |  |
| *Aerococcus* | 7.25E-07 | 4.15E-06 | 4.36E-06 | 1.75E-06 | 2.89E-06 | 1.30E-05 | 0 | 0 |  |  |
| *Agrobacterium* | 2.82E-05 | 2.65E-05 | 9.47E-06 | 2.45E-05 | 0.0007 | 2.00E-05 | 1.20E-05 | 0.0005 |  |  |
| *Agrococcus* | 0 | 1.04E-06 | 0 | 0 | 0 | 1.63E-06 | 0 | 0 |  |  |
| *Agromyces* | 0 | 3.94E-06 | 1.43E-06 | 3.81E-06 | 1.59E-06 | 2.28E-06 | 0 | 0 |  |  |
| *Akkermansia* | 0 | 7.51E-07 | 0 | 0 | 2.11E-05 | 0 | 0 | 0 |  |  |
| *Anaerococcus* | 0 | 7.51E-07 | 1.21E-06 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Anaeroplasma* | 0.0073 | 0.0045 | 0.0082 | 0.0018 | 0.0032 | 0.0044 | 0.0040 | 0.0022 |  |  |
| *Anaerostipes* | 0.0003 | 0.0003 | 0.0003 | 0.0006 | 0.0002 | 9.42E-05 | 9.43E-05 | 3.52E-05 |  |  |
| *Anaerotruncus* | 0 | 3.19E-06 | 5.28E-06 | 1.81E-06 | 8.66E-06 | 1.15E-05 | 0 | 3.95E-06 |  |  |
| *Anaerovibrio* | 2.19E-05 | 7.29E-05 | 6.87E-05 | 0.0008 | 0.0002 | 0.0006 | 0.0001 | 0.0008 |  |  |
| *Arcobacter* | 0 | 0 | 0 | 0 | 5.80E-06 | 1.62E-05 | 8.70E-05 | 0.0002 |  |  |
| *Arthrobacter* | 0 | 4.23E-06 | 4.31E-06 | 8.76E-07 | 1.17E-05 | 7.16E-06 | 3.27E-06 | 2.17E-06 |  |  |
| *Asteroleplasma* | 0.0017 | 0.0011 | 0.0013 | 0.0014 | 0.0006 | 0.0010 | 0.0013 | 0.0009 |  |  |
| *Asticcacaulis* | 0 | 3.12E-06 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Atopobium* | 3.32E-06 | 9.17E-06 | 1.75E-05 | 4.58E-05 | 8.90E-05 | 2.63E-05 | 3.57E-05 | 0.0002 |  |  |
| *Aurantimonas* | 3.45E-06 | 7.20E-06 | 9.19E-07 | 2.96E-06 | 7.94E-07 | 6.02E-06 | 9.54E-07 | 9.88E-07 |  |  |
| *B-42* | 0 | 0 | 0 | 2.41E-06 | 0 | 0 | 0 | 3.09E-06 |  |  |
| *Bacillus* | 1.17E-05 | 3.54E-05 | 1.63E-05 | 6.41E-05 | 1.49E-05 | 8.66E-05 | 1.33E-05 | 0.0002 |  |  |
| *Bacteroides* | 0.0002 | 0.0001 | 0.0002 | 0.0004 | 0.0004 | 0.0009 | 0.0004 | 0.0032 |  |  |
| *Balneimonas* | 3.33E-06 | 2.08E-06 | 1.72E-06 | 8.88E-06 | 7.94E-07 | 3.12E-06 | 0 | 0 |  |  |
| *BF311* | 0.0006 | 0.0001 | 0.0011 | 4.44E-05 | 0.0055 | 0.0009 | 0.0124 | 0.0006 |  |  |
| *Bifidobacterium* | 0.0002 | 0.0001 | 3.04E-05 | 0.0015 | 1.06E-05 | 0.0001 | 5.41E-06 | 0.0002 |  |  |
| *Blautia* | 2.06E-05 | 1.88E-05 | 7.82E-05 | 4.32E-05 | 7.35E-05 | 3.16E-05 | 1.03E-05 | 1.31E-05 |  |  |
| *Brachybacterium* | 0 | 1.50E-06 | 0 | 0 | 0 | 6.31E-06 | 7.02E-06 | 0 |  |  |
| *Brevibacillus* | 0 | 0 | 0 | 2.41E-06 | 0 | 1.62E-06 | 0 | 0 |  |  |
| *Brevibacterium* | 0 | 0 | 1.45E-06 | 0 | 0 | 3.23E-06 | 0 | 0 |  |  |
| *Bulleidia* | 0.0005 | 0.0008 | 0.0005 | 0.0044 | 0.0004 | 0.0007 | 0.0002 | 0.0007 |  |  |
| *Butyricimonas* | 1.39E-05 | 4.42E-06 | 2.88E-05 | 5.34E-05 | 5.40E-05 | 1.31E-05 | 0.0002 | 9.53E-05 |  |  |
| *Butyrivibrio* | 0.0160 | 0.0140 | 0.0115 | 0.0161 | 0.0044 | 0.0037 | 0.0054 | 0.0033 | *\** |  |
| *Campylobacter* | 7.31E-05 | 5.33E-05 | 0.0001 | 0.0003 | 0.0002 | 0.0015 | 0.0004 | 0.0017 |  |  |
| *CandidatusArthromitus* | 1.79E-06 | 0 | 0 | 0 | 0 | 0 | 9.54E-07 | 0 |  |  |
| *Carnobacterium* | 0 | 0 | 1.45E-06 | 7.89E-06 | 0 | 0 | 0 | 0 |  |  |
| *Cellulosimicrobium* | 2.18E-06 | 4.62E-06 | 0 | 0 | 0 | 1.47E-06 | 0 | 0 |  |  |
| *Cellvibrio* | 0 | 2.08E-06 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *CF231* | 0.0060 | 0.0057 | 0.0117 | 0.0029 | 0.0100 | 0.0158 | 0.0207 | 0.0113 |  |  |
| *Christensenella* | 2.10E-05 | 1.33E-05 | 1.55E-05 | 2.03E-05 | 3.03E-05 | 5.48E-06 | 1.81E-05 | 1.67E-05 |  |  |
| *Chryseobacterium* | 3.72E-06 | 5.23E-06 | 2.02E-05 | 2.96E-06 | 5.74E-05 | 1.71E-05 | 0.0001 | 3.81E-05 |  |  |
| *Cloacibacterium* | 0 | 0 | 0 | 8.46E-07 | 7.41E-06 | 1.38E-05 | 1.13E-05 | 3.96E-05 |  |  |
| *Clostridium* | 0.0016 | 0.0020 | 0.0192 | 0.0013 | 0.0072 | 0.0031 | 0.0052 | 0.0018 |  |  |
| *Comamonas* | 1.62E-05 | 2.56E-05 | 7.71E-06 | 7.64E-05 | 7.05E-05 | 0.0002 | 0.0011 | 0.0005 |  |  |
| *Coprococcus* | 0.0025 | 0.0033 | 0.0192 | 0.0023 | 0.0045 | 0.0087 | 0.0034 | 0.0006 |  |  |
| *Corallococcus* | 0 | 0 | 0 | 2.88E-06 | 0 | 0 | 0 | 0 |  |  |
| *Corynebacterium* | 9.77E-05 | 0.0003 | 9.37E-05 | 0.0004 | 0.0003 | 0.0005 | 0.0002 | 0.0006 |  |  |
| *Cryocola* | 0 | 2.25E-06 | 0 | 0 | 9.75E-07 | 8.16E-07 | 0 | 0 |  |  |
| *Curtobacterium* | 4.95E-06 | 4.36E-06 | 8.61E-07 | 5.75E-06 | 3.72E-06 | 7.28E-06 | 1.63E-06 | 2.23E-06 |  |  |
| *Dechloromonas* | 0 | 0 | 0 | 0 | 9.63E-07 | 3.62E-06 | 6.62E-06 | 2.72E-05 |  |  |
| *Dehalobacterium* | 5.59E-06 | 8.60E-06 | 0.0001 | 1.61E-05 | 0.0002 | 3.13E-05 | 9.60E-05 | 3.53E-06 |  |  |
| *Delftia* | 5.27E-06 | 4.23E-06 | 8.61E-07 | 2.63E-06 | 0.0005 | 4.55E-06 | 6.99E-06 | 0.0002 |  |  |
| *Desulfobulbus* | 3.09E-05 | 1.71E-05 | 3.74E-05 | 0.0002 | 0.0002 | 0.0006 | 0.0001 | 0.0010 |  |  |
| *Desulfovibrio* | 4.69E-05 | 6.25E-05 | 7.70E-05 | 0.0003 | 0.0002 | 0.0001 | 0.0002 | 4.53E-05 |  |  |
| *Devosia* | 6.03E-06 | 2.17E-05 | 4.59E-06 | 1.30E-05 | 6.98E-06 | 1.96E-05 | 1.63E-06 | 9.04E-06 |  |  |
| *Dialister* | 0.0066 | 0.0072 | 0.0072 | 0.0171 | 0.0010 | 0.0001 | 0.0004 | 0.0007 |  |  |
| *Dorea* | 0 | 7.51E-07 | 1.45E-06 | 3.47E-06 | 1.00E-06 | 4.74E-06 | 1.01E-05 | 0 |  |  |
| *Dysgonomonas* | 7.25E-07 | 1.31E-05 | 9.19E-07 | 1.75E-06 | 2.93E-06 | 4.55E-06 | 1.77E-06 | 6.97E-05 |  |  |
| *Elusimicrobium* | 0.0002 | 0.0015 | 1.44E-05 | 1.81E-06 | 3.45E-05 | 0.0001 | 0.0002 | 6.62E-06 |  |  |
| *Enterococcus* | 1.85E-06 | 0 | 7.03E-06 | 9.77E-06 | 5.27E-06 | 1.12E-05 | 2.83E-06 | 0.0001 |  |  |
| *Epulopiscium* | 0 | 0 | 0 | 0 | 0 | 0 | 1.91E-06 | 0 |  |  |
| *Erysipelothrix* | 3.96E-07 | 9.57E-06 | 7.27E-07 | 0 | 1.08E-05 | 1.32E-05 | 6.10E-06 | 7.30E-06 |  |  |
| *Exiguobacterium* | 0 | 0 | 0 | 0 | 0 | 0 | 2.72E-06 | 0 |  |  |
| *Facklamia* | 5.62E-06 | 6.44E-05 | 1.96E-05 | 7.85E-06 | 5.42E-05 | 3.08E-05 | 3.40E-05 | 5.08E-05 |  |  |
| *Fibrobacter* | 0.0726 | 0.0176 | 0.1198 | 0.0012 | 0.0128 | 0.0089 | 0.0839 | 0.0209 |  |  |
| *Filifactor* | 2.98E-06 | 0 | 0 | 0 | 0 | 0 | 1.77E-06 | 0 |  |  |
| *Flavobacterium* | 3.96E-07 | 7.51E-07 | 0 | 0 | 4.85E-06 | 8.02E-06 | 1.92E-05 | 4.46E-05 |  |  |
| *Friedmanniella* | 0 | 0 | 2.30E-06 | 8.76E-07 | 1.59E-06 | 0 | 0 | 0 |  |  |
| *Fusobacterium* | 1.95E-05 | 2.14E-05 | 3.53E-05 | 2.04E-05 | 7.54E-05 | 0.0007 | 0.0002 | 0.0016 |  |  |
| *Gallicola* | 0 | 1.04E-06 | 4.21E-06 | 3.01E-06 | 2.00E-06 | 7.34E-06 | 0 | 0 |  |  |
| *Glycomyces* | 4.48E-06 | 1.02E-05 | 0 | 2.96E-05 | 2.93E-06 | 3.22E-05 | 0 | 6.88E-06 |  |  |
| *Gordonia* | 0 | 1.04E-06 | 0 | 9.36E-07 | 0 | 0 | 0 | 9.88E-07 |  |  |
| *GW-34* | 1.58E-06 | 1.11E-06 | 0 | 0 | 1.59E-06 | 4.74E-06 | 0 | 1.91E-05 |  |  |
| *Helcococcus* | 4.70E-07 | 7.51E-07 | 0 | 1.81E-06 | 0 | 1.25E-05 | 8.80E-06 | 2.96E-06 |  |  |
| *Herbaspirillum* | 9.39E-07 | 2.08E-06 | 9.19E-07 | 0 | 0 | 0 | 0 | 1.12E-06 |  |  |
| *Hydrogenophaga* | 0 | 0 | 7.27E-07 | 3.62E-06 | 1.95E-06 | 1.47E-06 | 1.55E-05 | 0.0001 |  |  |
| *Hyphomicrobium* | 9.39E-07 | 0 | 0 | 4.09E-06 | 0 | 7.04E-06 | 0 | 0 |  |  |
| *Jeotgalicoccus* | 3.96E-07 | 1.11E-05 | 6.62E-06 | 7.55E-06 | 1.39E-05 | 9.79E-06 | 1.63E-06 | 5.63E-05 |  |  |
| *Jiangella* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Kaistobacter* | 0 | 0 | 0 | 8.76E-07 | 9.63E-07 | 0 | 0 | 0 |  |  |
| *Kocuria* | 0 | 4.50E-06 | 0 | 1.81E-06 | 1.00E-06 | 5.23E-06 | 0 | 2.10E-06 |  |  |
| *L7A\_E11* | 0.0002 | 5.84E-05 | 0.0001 | 0.0002 | 0.0009 | 0.0001 | 0.0013 | 0.0003 |  |  |
| *Lachnospira* | 0.0001 | 0.0003 | 0.0002 | 0.0006 | 9.63E-07 | 5.55E-06 | 1.05E-05 | 6.50E-06 |  |  |
| *Lactobacillus* | 0.0001 | 0.0014 | 0.0006 | 0.0015 | 3.78E-05 | 0.0012 | 9.98E-05 | 0.0013 |  |  |
| *Lactococcus* | 7.25E-07 | 0 | 9.19E-07 | 2.29E-05 | 2.55E-06 | 1.30E-05 | 0 | 8.35E-05 |  |  |
| *Lampropedia* | 0 | 3.12E-06 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Lautropia* | 0 | 4.15E-06 | 0 | 8.76E-07 | 5.56E-06 | 4.55E-06 | 8.11E-06 | 9.88E-07 |  |  |
| *Legionella* | 3.76E-06 | 0 | 0 | 0 | 0 | 0 | 0 | 1.12E-06 |  |  |
| *Leucobacter* | 1.92E-06 | 0 | 1.65E-06 | 8.99E-06 | 2.97E-06 | 0 | 9.54E-07 | 4.49E-05 |  |  |
| *Leuconostoc* | 0 | 0 | 1.43E-06 | 8.76E-07 | 3.93E-06 | 0 | 0 | 0 |  |  |
| *Limnohabitans* | 0 | 3.12E-06 | 0 | 1.95E-05 | 0 | 5.43E-06 | 9.25E-06 | 2.48E-05 |  |  |
| *Loktanella* | 0 | 0 | 9.19E-07 | 1.81E-06 | 0 | 0 | 0 | 0 |  |  |
| *Luteimonas* | 0 | 4.23E-06 | 1.45E-06 | 1.40E-05 | 0 | 8.97E-06 | 1.91E-06 | 1.14E-05 |  |  |
| *Lutibacterium* | 0 | 0 | 0 | 1.00E-06 | 0 | 0 | 0 | 0 |  |  |
| *Lysinibacillus* | 4.62E-06 | 1.11E-06 | 1.45E-06 | 5.92E-06 | 0.0005 | 1.47E-06 | 2.39E-05 | 8.13E-05 |  |  |
| *Lysobacter* | 1.88E-06 | 0 | 2.16E-06 | 3.82E-06 | 0 | 1.47E-06 | 5.25E-06 | 0 |  |  |
| *Macrococcus* | 4.70E-07 | 7.51E-07 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Megasphaera* | 0.0013 | 0.0005 | 0.0017 | 0.0032 | 0.0005 | 0.0065 | 0.0008 | 0.0883 | *\** |  |
| *Methanobrevibacter* | 1.92E-06 | 0 | 0 | 7.22E-06 | 7.94E-07 | 0 | 0 | 0 |  |  |
| *Methylobacterium* | 2.65E-06 | 0 | 0 | 0 | 0.0001 | 1.47E-06 | 0 | 0 |  |  |
| *Microbacterium* | 3.34E-06 | 3.87E-06 | 6.37E-06 | 2.16E-05 | 5.80E-06 | 7.52E-06 | 0 | 2.97E-05 |  |  |
| *Micrococcus* | 3.96E-07 | 1.04E-06 | 0 | 0 | 4.35E-06 | 0 | 0 | 4.08E-06 |  |  |
| *Mitsuokella* | 0.0005 | 0.0004 | 0.0006 | 0.0020 | 0.0002 | 0.0012 | 0.0002 | 0.0021 |  |  |
| *Mogibacterium* | 1.38E-05 | 1.54E-05 | 1.34E-05 | 5.43E-05 | 6.98E-05 | 3.03E-05 | 5.52E-05 | 3.19E-05 |  |  |
| *Moraxella* | 0 | 0 | 3.10E-06 | 1.21E-06 | 0.0001 | 8.32E-06 | 5.28E-06 | 3.34E-06 |  |  |
| *Morganella* | 0 | 1.11E-06 | 0 | 0 | 0 | 0 | 0 | 1.18E-06 |  |  |
| *Moryella* | 0.0006 | 0.0006 | 0.0004 | 0.0007 | 0.0051 | 0.0013 | 0.0011 | 0.0003 |  |  |
| *Mucispirillum* | 3.29E-06 | 0 | 0 | 0 | 1.59E-06 | 0 | 9.80E-06 | 5.58E-06 |  |  |
| *Mycobacterium* | 4.99E-06 | 0 | 8.61E-07 | 1.28E-05 | 7.56E-06 | 0 | 4.90E-06 | 5.97E-05 |  |  |
| *Mycoplana* | 2.73E-06 | 8.02E-06 | 9.19E-07 | 1.82E-05 | 5.01E-06 | 0 | 4.90E-06 | 6.69E-06 |  |  |
| *Myroides* | 0 | 0 | 0 | 0 | 1.00E-06 | 5.87E-06 | 2.86E-06 | 0 |  |  |
| *Natronobacillus* | 0 | 0 | 9.19E-07 | 9.36E-07 | 0 | 0 | 0 | 0 |  |  |
| *Neisseria* | 0 | 0 | 0 | 0 | 0 | 0 | 4.57E-06 | 0 |  |  |
| *Nesterenkonia* | 0 | 0 | 0 | 0 | 0 | 8.16E-07 | 0 | 0 |  |  |
| *Nocardioides* | 0 | 1.04E-06 | 0 | 0 | 9.75E-07 | 0 | 0 | 0 |  |  |
| *Nocardiopsis* | 0 | 7.51E-07 | 0 | 1.00E-06 | 0 | 1.56E-06 | 0 | 1.18E-06 |  |  |
| *Novosphingobium* | 0 | 1.04E-06 | 0 | 0 | 0 | 0 | 0 | 9.88E-07 |  |  |
| *Ochrobactrum* | 0 | 1.04E-06 | 0 | 1.00E-06 | 0 | 0 | 0 | 0 |  |  |
| *Odoribacter* | 3.84E-06 | 0 | 0 | 0 | 4.21E-05 | 0 | 0 | 0 |  |  |
| *Oligella* | 0 | 3.87E-06 | 2.56E-06 | 1.00E-06 | 0 | 0 | 1.74E-06 | 0 |  |  |
| *Olivibacter* | 0 | 0 | 0 | 1.85E-06 | 0 | 0 | 0 | 0 |  |  |
| *Oribacterium* | 0 | 0 | 0 | 0 | 0 | 0 | 5.25E-06 | 9.88E-07 |  |  |
| *Oscillospira* | 0.0058 | 0.0094 | 0.0032 | 0.0028 | 0.0021 | 0.0046 | 0.0025 | 0.0009 |  |  |
| *p-75-a5* | 0.0004 | 0.0003 | 0.0006 | 0.0011 | 0.0013 | 0.0004 | 0.0015 | 0.0004 |  |  |
| *Paenibacillus* | 3.44E-06 | 1.37E-05 | 3.05E-06 | 5.14E-05 | 1.59E-06 | 2.00E-05 | 1.74E-06 | 0 |  |  |
| *Paludibacter* | 2.31E-06 | 0 | 0 | 0 | 9.63E-07 | 0 | 2.86E-06 | 0.0001 |  |  |
| *Parabacteroides* | 0.0001 | 0.0002 | 3.01E-05 | 0.0003 | 0.0002 | 0.0002 | 9.29E-05 | 0.0002 |  |  |
| *Paracoccus* | 1.33E-06 | 2.08E-06 | 0 | 8.10E-06 | 1.59E-06 | 8.45E-06 | 9.54E-07 | 1.98E-06 |  |  |
| *Parvimonas* | 0 | 3.12E-06 | 0 | 0 | 1.00E-06 | 0 | 3.53E-06 | 1.98E-06 |  |  |
| *Patulibacter* | 1.80E-06 | 1.50E-06 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Peptoniphilus* | 0 | 5.94E-06 | 0 | 0 | 9.75E-07 | 1.47E-06 | 0 | 0.0001 |  |  |
| *Peptostreptococcus* | 0 | 1.11E-06 | 0 | 0 | 0 | 0 | 0 | 1.18E-06 |  |  |
| *ph2* | 0 | 0 | 0 | 0 | 2.38E-06 | 2.94E-06 | 0 | 0 |  |  |
| *Phascolarctobacterium* | 7.52E-06 | 1.44E-05 | 0.0006 | 0.0002 | 8.01E-06 | 3.28E-06 | 3.73E-05 | 2.67E-05 |  |  |
| *Phenylobacterium* | 2.13E-06 | 8.31E-06 | 1.59E-06 | 0 | 0 | 0 | 1.74E-06 | 1.12E-06 |  |  |
| *Phycicoccus* | 0 | 0 | 0 | 1.75E-06 | 0 | 1.81E-06 | 0 | 0 |  |  |
| *Planomicrobium* | 8.65E-07 | 0 | 0 | 0 | 4.59E-06 | 0 | 0 | 0 |  |  |
| *Polynucleobacter* | 3.96E-07 | 0 | 3.02E-06 | 6.91E-06 | 9.75E-07 | 8.05E-06 | 5.67E-06 | 1.15E-05 |  |  |
| *Pontibacter* | 9.39E-07 | 0 | 0 | 0 | 0 | 0 | 2.83E-06 | 0 |  |  |
| *Porphyromonas* | 7.54E-06 | 1.62E-05 | 1.97E-05 | 3.84E-05 | 6.65E-05 | 2.76E-05 | 0.0005 | 0.0001 |  |  |
| *Prauserella* | 4.01E-06 | 3.00E-06 | 2.64E-06 | 7.97E-06 | 0 | 7.13E-06 | 6.96E-06 | 2.23E-06 |  |  |
| *Prauseria* | 1.05E-05 | 6.89E-06 | 7.48E-06 | 1.46E-05 | 7.90E-06 | 1.45E-05 | 1.33E-05 | 2.32E-05 |  |  |
| *Prevotella* | 0.4305 | 0.4253 | 0.2603 | 0.2649 | 0.1258 | 0.1841 | 0.1431 | 0.2218 | *\** |  |
| *Propionibacterium* | 7.67E-06 | 1.28E-05 | 0 | 9.92E-06 | 6.35E-06 | 1.34E-05 | 7.62E-06 | 0.0001 |  |  |
| *Proteiniclasticum* | 0 | 0 | 0 | 0 | 1.95E-06 | 8.16E-07 | 0 | 9.88E-07 |  |  |
| *Proteus* | 0 | 0 | 0 | 0 | 0 | 0 | 9.54E-07 | 1.01E-05 |  |  |
| *Providencia* | 0 | 0 | 0 | 0 | 0 | 0 | 1.74E-06 | 1.12E-06 |  |  |
| *Pseudobutyrivibrio* | 0.0021 | 0.0009 | 0.0010 | 0.0005 | 0.0005 | 0.0011 | 0.0002 | 3.17E-05 |  |  |
| *Pseudomonas* | 3.96E-07 | 5.66E-06 | 0 | 3.38E-05 | 0.0001 | 8.31E-05 | 8.31E-05 | 0.0002 |  |  |
| *Pseudonocardia* | 0 | 2.83E-06 | 1.59E-06 | 3.44E-06 | 3.73E-06 | 9.80E-06 | 0 | 9.27E-06 |  |  |
| *Pseudoramibacter* | 0.0005 | 0.0004 | 0.0003 | 0.0040 | 0.0006 | 0.0022 | 0.0001 | 0.0004 |  |  |
| *Pseudoxanthomonas* | 0 | 1.04E-06 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |
| *Psychrobacter* | 2.64E-06 | 9.57E-06 | 2.91E-06 | 1.69E-06 | 3.72E-06 | 1.78E-05 | 1.58E-05 | 1.98E-05 |  |  |
| *Pyramidobacter* | 0.0004 | 0.0003 | 0.0005 | 0.0002 | 0.0009 | 0.0004 | 0.0020 | 0.0013 |  |  |
| *RFN20* | 0.0122 | 0.0055 | 0.0089 | 0.0044 | 0.0324 | 0.0058 | 0.0401 | 0.0067 |  |  |
| *Rheinheimera* | 0 | 0 | 7.27E-07 | 0 | 0 | 4.40E-06 | 1.77E-06 | 6.37E-06 |  |  |
| *Rhizobium* | 2.65E-06 | 2.08E-06 | 0 | 0 | 0.0002 | 1.62E-06 | 0 | 0.0001 |  |  |
| *Rhodobacter* | 0 | 3.12E-06 | 8.61E-07 | 1.64E-05 | 0 | 3.62E-06 | 3.79E-06 | 4.58E-06 |  |  |
| *Rhodococcus* | 0 | 3.12E-06 | 8.61E-07 | 1.00E-06 | 9.75E-07 | 4.19E-06 | 1.63E-06 | 4.33E-06 |  |  |
| *Rickettsiella* | 0 | 0 | 0 | 5.03E-06 | 0 | 0 | 0 | 0 |  |  |
| *Roseburia* | 0.0040 | 0.0117 | 0.0013 | 0.0045 | 4.76E-05 | 0.0001 | 4.08E-05 | 2.38E-05 |  |  |
| *Rubellimicrobium* | 4.70E-07 | 0 | 0 | 0 | 9.63E-07 | 2.94E-06 | 9.54E-07 | 0 |  |  |
| *Rubrobacter* | 0 | 0 | 0 | 0 | 7.94E-07 | 1.47E-06 | 0 | 0 |  |  |
| *Ruminobacter* | 9.14E-05 | 7.79E-05 | 0.0002 | 0.0004 | 0.0002 | 0.0025 | 0.0010 | 0.0028 | *\** |  |
| *Ruminococcus* | 1.65E-02 | 2.28E-02 | 3.07E-02 | 2.39E-02 | 1.52E-02 | 1.99E-02 | 8.96E-03 | 3.57E-03 | *\** |  |
| *Rummeliibacillus* | 2.13E-06 | 1.36E-05 | 7.32E-05 | 4.63E-06 | 0.0001 | 2.04E-05 | 3.42E-05 | 4.94E-06 |  |  |
| *Saccharomonospora* | 0 | 7.51E-07 | 0 | 2.01E-06 | 9.75E-07 | 4.09E-06 | 0 | 7.42E-06 |  |  |
| *Saccharopolyspora* | 2.45E-05 | 2.89E-05 | 1.93E-05 | 6.90E-05 | 1.57E-05 | 7.13E-05 | 7.27E-06 | 8.27E-05 |  |  |
| *Salinispora* | 0 | 0 | 0 | 0 | 0 | 1.62E-06 | 0 | 0 |  |  |
| *Sedimentibacter* | 0 | 0 | 7.27E-07 | 0 | 0 | 3.10E-06 | 5.22E-06 | 1.01E-05 |  |  |
| *Selenomonas* | 4.40E-05 | 7.71E-05 | 0.0002 | 0.0072 | 0.0001 | 0.0008 | 7.26E-05 | 0.0003 |  |  |
| *Sharpea* | 0.0099 | 0.0667 | 0.0055 | 0.0850 | 0.0045 | 0.0883 | 0.0008 | 0.0149 |  |  |
| *SHD-231* | 0 | 1.81E-06 | 0 | 1.00E-06 | 1.90E-05 | 8.97E-06 | 1.74E-05 | 3.95E-06 |  |  |
| *Shuttleworthia* | 0.0014 | 0.0030 | 0.0036 | 0.0029 | 0.0001 | 0.0001 | 4.59E-05 | 1.55E-05 |  |  |
| *Skermanella* | 0 | 0 | 0 | 0 | 9.75E-07 | 4.40E-06 | 0 | 2.35E-06 |  |  |
| *SMB53* | 0 | 0 | 8.61E-07 | 1.81E-06 | 7.94E-07 | 1.47E-06 | 0 | 0 |  |  |
| *Solibacillus* | 0 | 0 | 7.27E-07 | 0 | 0 | 1.47E-06 | 0 | 0 |  |  |
| *Sphaerochaeta* | 0.0139 | 0.0068 | 0.0039 | 0.0034 | 0.0046 | 0.0078 | 0.0050 | 0.0036 |  |  |
| *Sphingobacterium* | 1.06E-06 | 6.69E-06 | 6.39E-06 | 2.69E-05 | 4.01E-06 | 1.79E-05 | 1.16E-05 | 8.48E-06 |  |  |
| *Sphingobium* | 1.06E-06 | 3.12E-06 | 0 | 0 | 0 | 1.62E-06 | 0 | 1.18E-06 |  |  |
| *Sphingomonas* | 3.79E-06 | 6.12E-06 | 4.94E-06 | 7.02E-06 | 0.0336 | 2.48E-05 | 6.34E-06 | 9.15E-06 |  |  |
| *Sphingopyxis* | 0 | 0 | 0 | 0 | 0 | 3.62E-06 | 0 | 2.35E-06 |  |  |
| *Sporosarcina* | 0 | 0 | 0 | 0 | 1.00E-06 | 2.38E-06 | 9.54E-07 | 9.88E-07 |  |  |
| *Staphylococcus* | 7.88E-06 | 1.89E-05 | 1.33E-05 | 5.49E-05 | 1.96E-05 | 7.26E-05 | 8.22E-06 | 0.0002 |  |  |
| *Stenotrophomonas* | 6.80E-06 | 1.34E-05 | 9.90E-06 | 7.50E-05 | 4.01E-06 | 1.22E-05 | 3.67E-05 | 0.0003 |  |  |
| *Steroidobacter* | 0 | 0 | 0 | 1.00E-06 | 0 | 8.16E-07 | 0 | 0 |  |  |
| *Streptococcus* | 0.0002 | 0.0002 | 0.0003 | 0.0132 | 0.0020 | 0.2404 | 0.0004 | 0.3625 | *\** | *\** |
| *Streptomyces* | 1.25E-05 | 1.52E-05 | 5.57E-06 | 3.74E-05 | 9.56E-06 | 6.23E-05 | 1.20E-05 | 9.58E-05 |  |  |
| *Succiniclasticum* | 0.0102 | 0.0162 | 0.0183 | 0.0144 | 0.0198 | 0.0166 | 0.0195 | 0.0130 |  |  |
| *Succinivibrio* | 0.0008 | 0.0009 | 0.0009 | 0.0009 | 0.0001 | 0.0013 | 0.0002 | 0.0005 |  |  |
| *Sutterella* | 0.0005 | 0.0006 | 0.0007 | 0.0002 | 0.0002 | 0.0006 | 0.0006 | 0.0006 |  |  |
| *Synergistes* | 0 | 0 | 9.19E-07 | 0 | 7.01E-06 | 0 | 3.81E-06 | 0 |  |  |
| *Tatlockia* | 0 | 2.72E-06 | 0 | 1.00E-06 | 0 | 0 | 0 | 0 |  |  |
| *Tessaracoccus* | 0 | 1.04E-06 | 0 | 8.46E-07 | 2.38E-06 | 1.47E-06 | 0 | 0 |  |  |
| *Tetrasphaera* | 0 | 0 | 0 | 0 | 0 | 0 | 1.74E-06 | 9.88E-07 |  |  |
| *TG5* | 1.03E-05 | 1.66E-05 | 6.77E-06 | 7.04E-05 | 7.56E-05 | 9.79E-05 | 0.0002 | 0.0003 |  |  |
| *Thauera* | 0 | 0 | 0 | 0 | 0 | 0 | 1.77E-06 | 1.12E-06 |  |  |
| *Tissierella\_Soehngenia* | 0 | 0 | 2.91E-06 | 0 | 0 | 0 | 2.83E-06 | 5.32E-06 |  |  |
| *Treponema* | 0.0128 | 0.0102 | 0.0343 | 0.0106 | 0.0141 | 0.0098 | 0.0219 | 0.0052 |  |  |
| *Trichococcus* | 9.52E-06 | 4.24E-05 | 2.53E-05 | 8.01E-06 | 4.72E-05 | 5.48E-05 | 3.32E-05 | 8.03E-05 |  |  |
| *Turicibacter* | 3.30E-05 | 5.30E-05 | 1.48E-05 | 3.15E-05 | 2.10E-05 | 6.58E-05 | 1.74E-06 | 0.0002 |  |  |
| *Ureibacillus* | 0 | 0 | 0 | 0 | 7.94E-07 | 3.23E-06 | 0 | 0 |  |  |
| *Veillonella* | 0 | 1.11E-06 | 7.27E-07 | 2.81E-06 | 0 | 4.40E-06 | 0 | 1.06E-05 |  |  |
| *Vogesella* | 0 | 0 | 0 | 0 | 0 | 3.23E-05 | 5.30E-06 | 1.18E-05 |  |  |
| *Wautersiella* | 4.10E-06 | 3.44E-06 | 1.53E-05 | 0.0004 | 5.85E-06 | 4.61E-05 | 2.58E-05 | 0.0002 |  |  |
| *Weeksella* | 1.19E-06 | 1.04E-06 | 0 | 1.21E-06 | 0 | 0 | 0 | 9.88E-07 |  |  |
| *Weissella* | 9.34E-06 | 5.62E-06 | 1.23E-05 | 4.26E-05 | 6.06E-06 | 1.62E-06 | 0 | 0 |  |  |
| *Wohlfahrtiimonas* | 0 | 0 | 0 | 0 | 9.63E-07 | 1.62E-06 | 1.91E-06 | 0 |  |  |
| *YRC22* | 0.0123 | 0.0047 | 0.0154 | 0.0025 | 0.0037 | 0.0096 | 0.0060 | 0.0026 |  |  |
| Other  | 0.3422 | 0.3495 | 0.4031 | 0.4834 | 0.6758 | 0.3359 | 0.5997 | 0.2064 |   |   |

1 CON = no feed withdrawal; T12 = 12 h of feed withdrawal; T24 = 24 h of feed withdrawal; T36 = 36 h of feed withdrawal; FR: reintroduction of feed; 12 h-FR: 12 hours after feed reintroduction. FW = effect of treatment (feed withdrawal); T = effect of time; \* *P* < 0.05.