

# **Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges presented for the identification of performance enhancing probiotic bacteria**

**Running Title:** Microbiota and broiler performance

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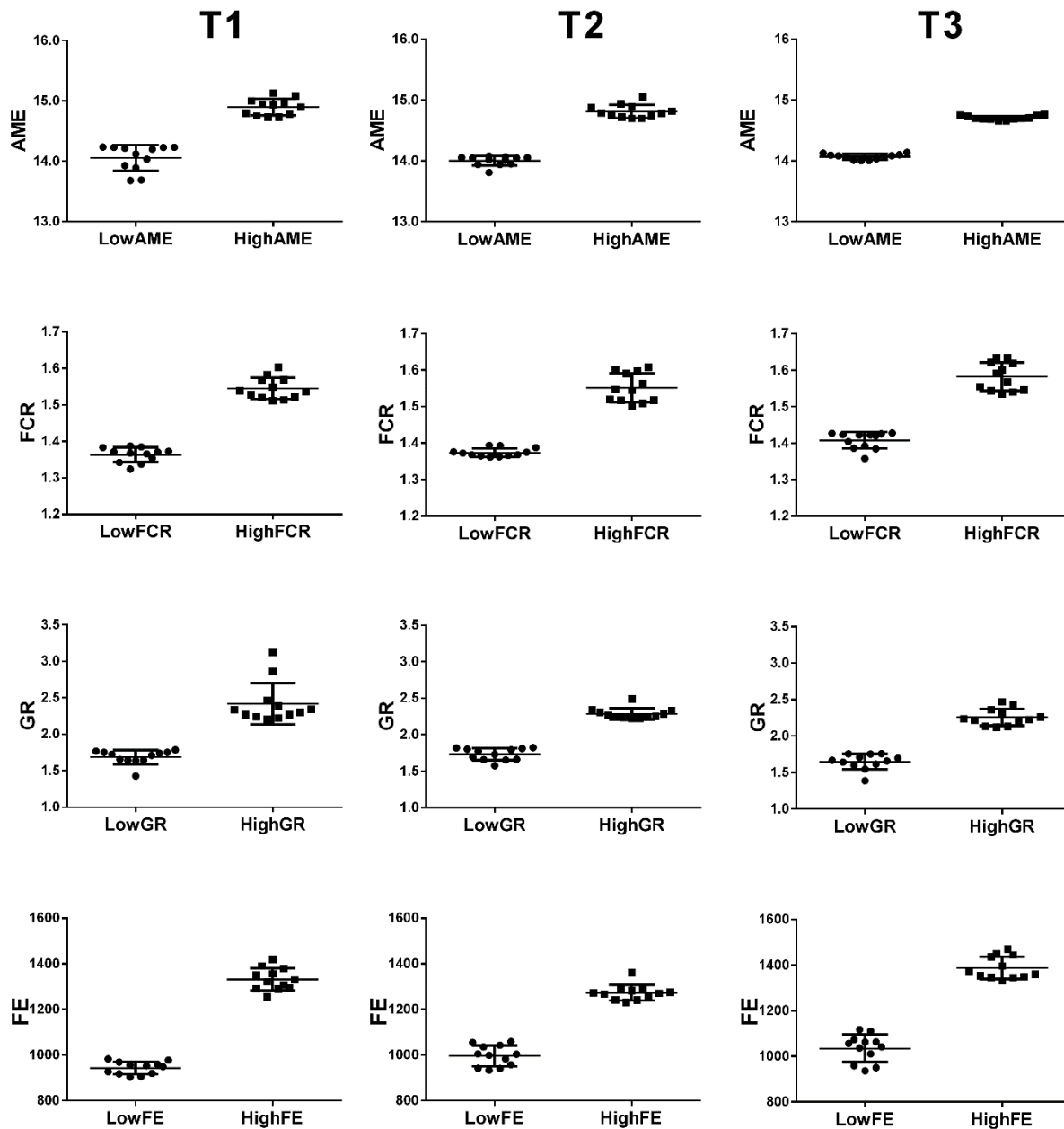
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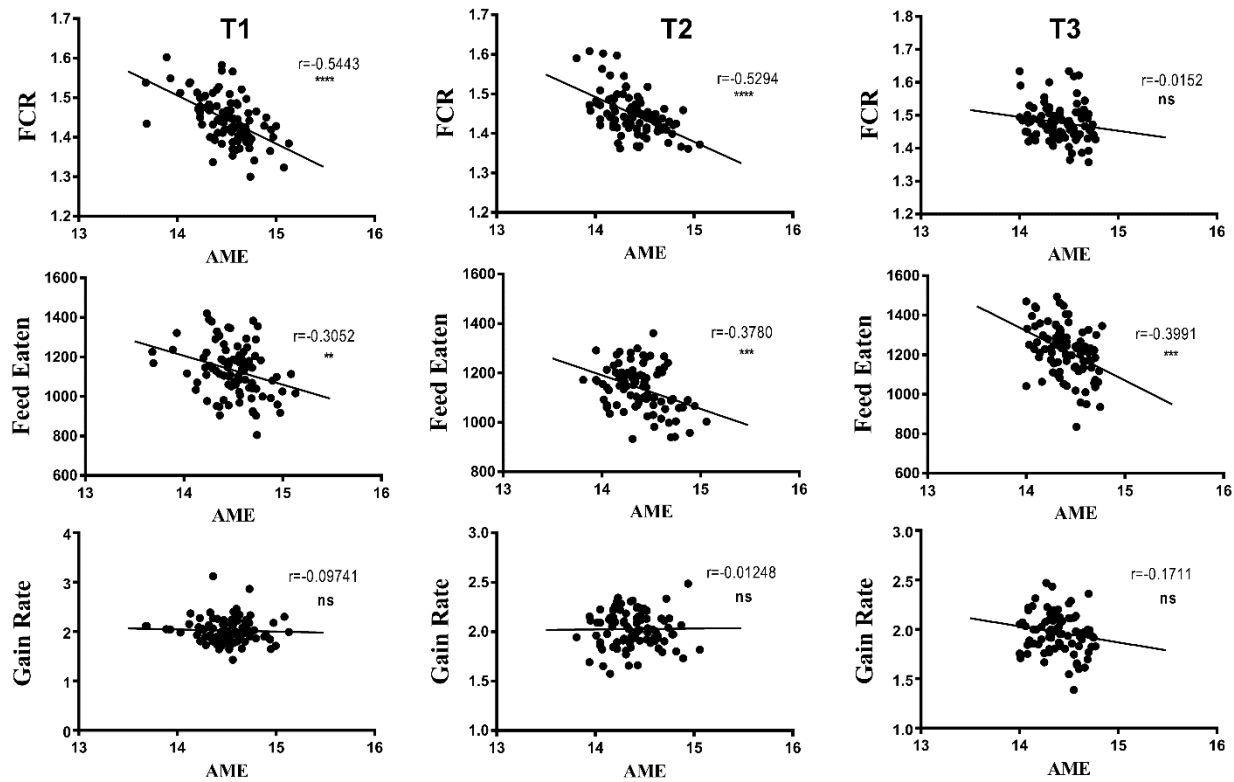
## Supplementary Figures

Figure S1



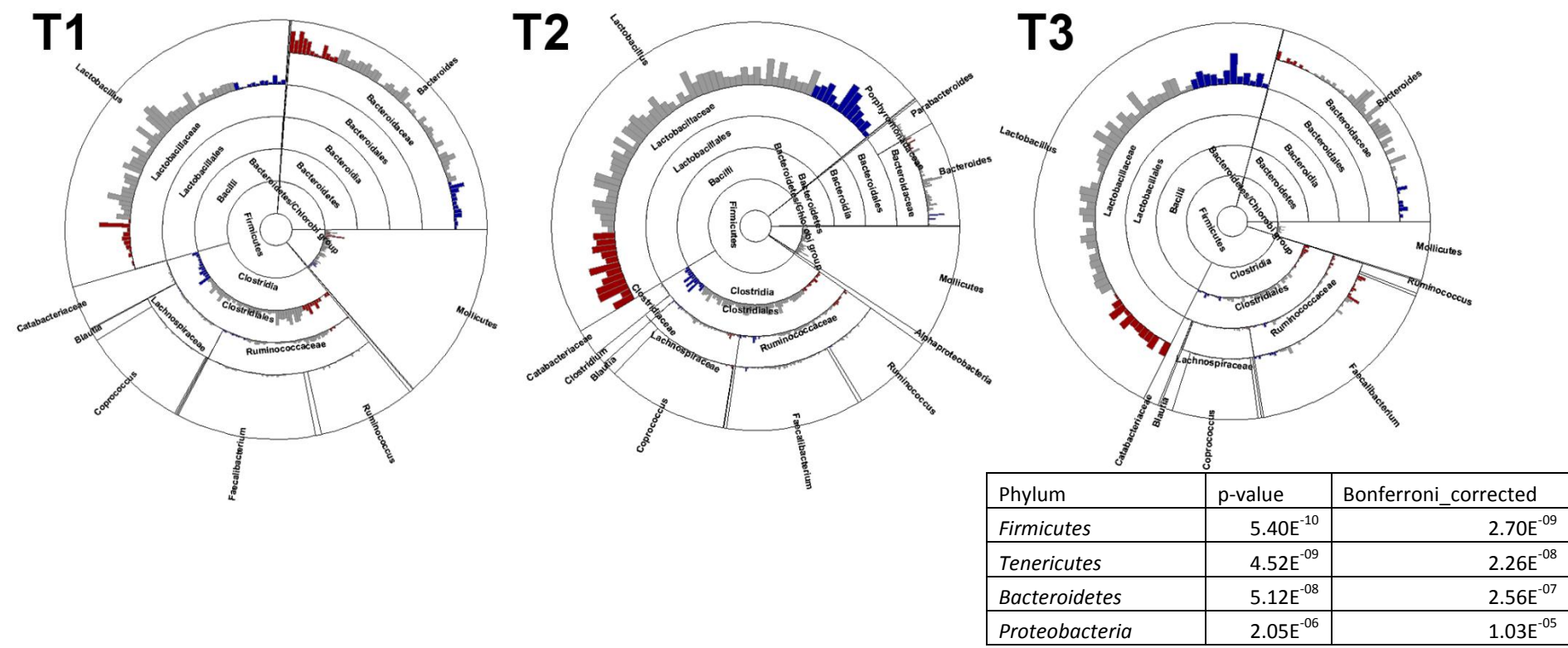
**Figure S1| Best and worst performing 12 birds of the flock.** The birds were differential in AME, FCR, GR and FE based on nonparametric Mann-Whitney U test, in all 3 trials (T1-T3). All p-values were  $<0.0001$ .

**Figure S2**



**Figure S2 | Correlations of AME against FCR, FE and GR.** The three trials are given in separate columns, Trial 1 in the left column, Trial 2 in the middle and T3 in the right column. The birds with higher AME values corresponding to better efficiency in energy extraction are likely to have lower (better) FCR, mostly due to eating less feed while having no change in gain rate. Figure was generated using animal trial data and GraphPad Prism software. P-value significance level and correlation coefficient (r) are based on Pearson correlation analysis between the two variables compared.

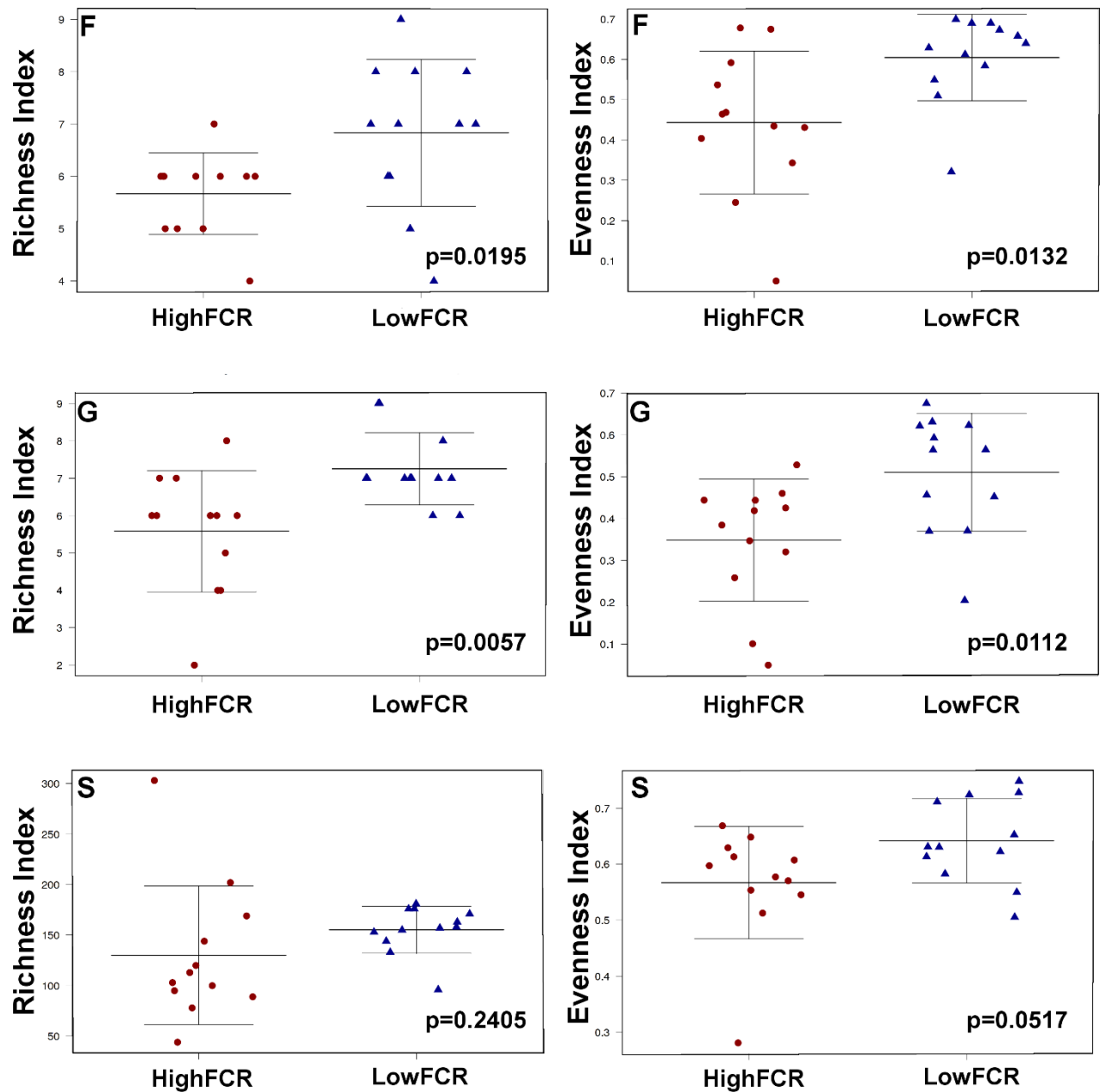
Figure S3



**Figure S3 | Taxonomy profile of the 3 trials shows significant differences at phylum level.** The graph was created by importing a QIIME biom table for each trial into MEGAN software (Huson et al., 2007). The size of the bars is proportional to the taxa abundance in each sample. Samples with

highest FCR are coloured red, lowest FCR blue and the remaining samples are grey. The three flocks were dominated by different phyla (Stanley et al., 2013).

FigureS4



**Figure S4 | Alpha diversity measures, Trial 1:** Alpha diversity, expressed as Richness and Evenness index, was differential between high and low FCR birds microbial communities at a family (row 1-labeled F) and genus (row 2 labelled G), but not at a species (OTU) level (row 3 labelled S).

Figure S5

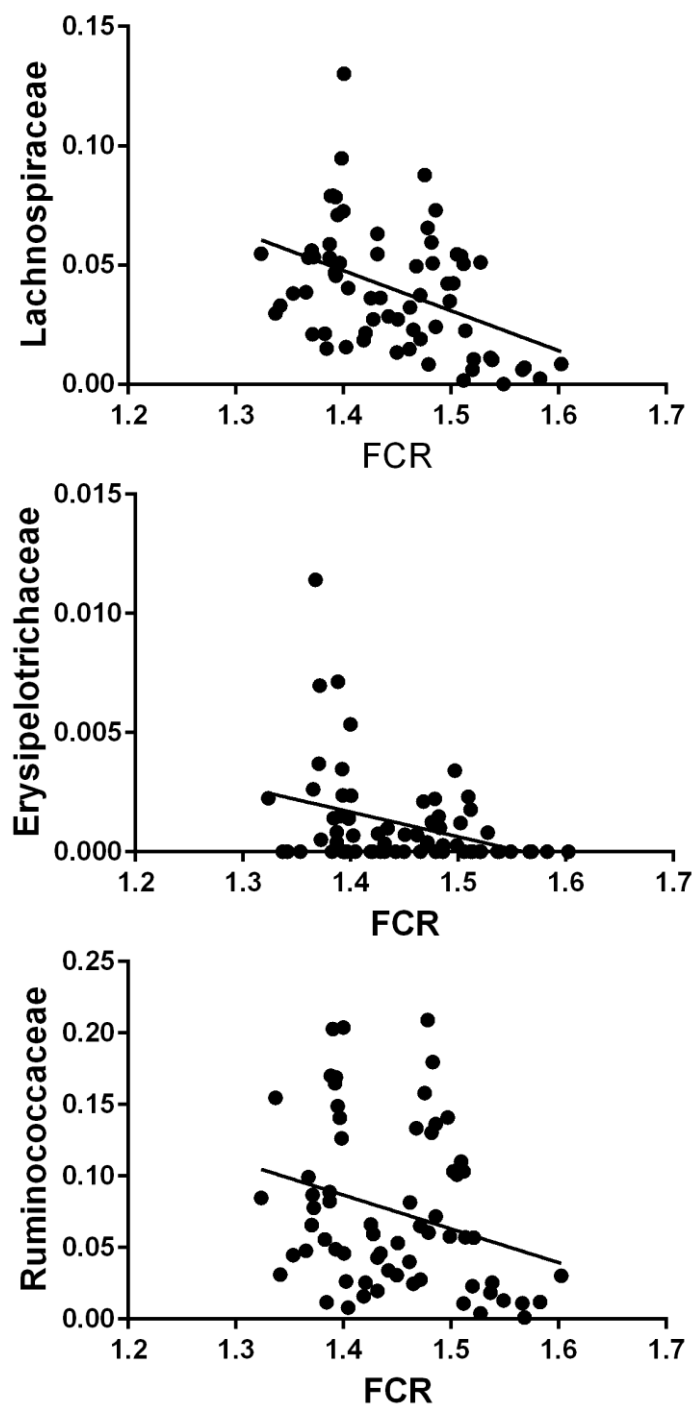
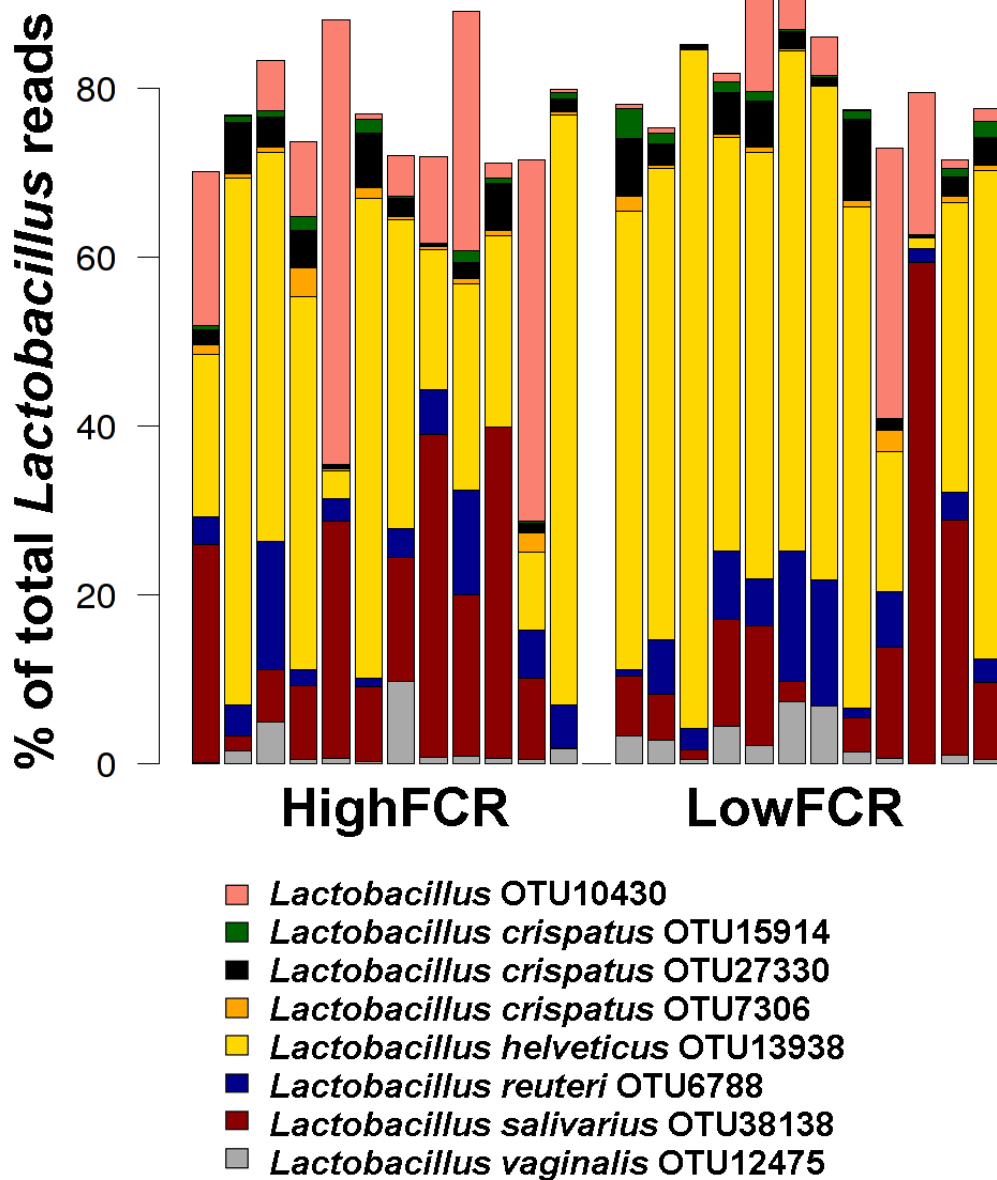


Figure S5 | Trial 1; three families showed significant negative Pearson correlation with FCR.

*Lachnospiraceae* ( $p=3.6E^{-4}$ ), *Erysipelotrichaceae* ( $p=5.0E^{-4}$ ) and *Ruminococcaceae* ( $p=0.0259$ ).

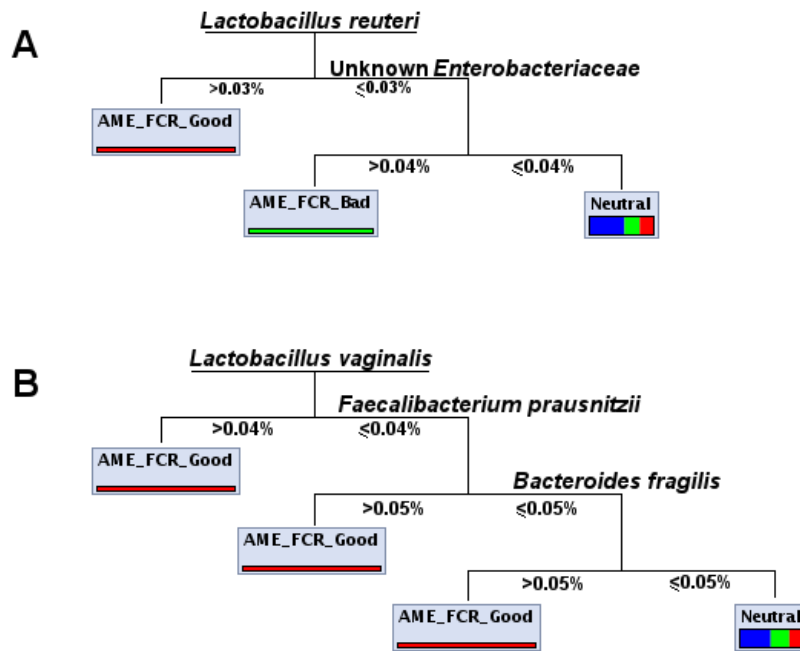
Figure S6



**Figure S6 | *Lactobacillus* profile shifts in high and low FCR birds in Trial 3.** There were 13 *Lactobacillus* OTUs more abundant ( $p < 0.05$ ) in good FCR birds and 5 others more abundant in poor FCR birds. Regardless of *Lactobacillus* OTUs dominating list of differential OTUs in trial 3, genus *Lactobacillus* was not significantly differentially abundant between high and low FCR birds indicating that shifts were occurring at the species level in both directions. In trial 2 *Lactobacillus* increased FCR by increasing feed eaten (Figure 8).



**Figure S7**



**Figure S7 | Random Forest data modelling.** A Random Forest algorithm was used to predict association of OTUs with birds that had both good AME and good FCR performance and also with undesirable bird trait - bad AME and bad FCR values. RapidMiner software was used to generate 50 predication trees, two of which are presented as A and B above. Each prediction was inspected and OTUs identified as associated with good performance included a number of OTUs identified as *Faecalibacterium prausnitzii*, *Bacteroides fragilis* and *Ruminococcus* species. Members of *Gammaproteobacteria* and genus *Clostridium* were repetitively associated with bad AME and bad FCR. Both these trees show *Lactobacillus* OTUs associated with good performance but other trees had members of the genus *Lactobacillus* whose abundance was positively predictive of bad performance.

## Supplementary Tables

Please note that supplementary tables present only data with  $p < 0.01$  due to the size of  $p < 0.05$  data for all 8 tables presented.

**Table S1** | Different levels of taxa significantly (QIIME t-test,  $p < 0.01$ ) differentially abundant between microbial communities sampled from the 12vs. 12 extreme birds based on FCR. High indicates that taxa is present only in high **FCR** birds.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                          | p-value  | FoldGood |
|----------------|------------------------------------------------------------------------------|----------|----------|
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae                          | 0.0001   | 3.41     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                          | 0.0005   | 3.22     |
| FAMILY         | Tenericutes/Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae           | 0.0263   | 36.57    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Clostridium</i>      | 8.04E-05 | 4.76     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i> | 0.0002   | 20.14    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/unknown                  | 0.0008   | 3.80     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/unknown                  | 0.0092   | 2.51     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus</i>     | 0.0132   | 3.83     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Ruminococcus</i>     | 0.0297   | 3.60     |
| denovo4873     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i> | 0.0009   | 22.00    |
| denovo13839    | Tenericutes/Mollicutes/RF39                                                  | 0.0011   | -3.89    |
| denovo18285    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i> | 0.0016   | 16.86    |
| denovo6476     | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Clostridium</i>      | 0.0044   | 15.80    |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                          | p-value  | FoldGood |

|                |                                                                                                   |          |          |
|----------------|---------------------------------------------------------------------------------------------------|----------|----------|
| FAMILY         | Firmicutes/Clostridia/Clostridiales/unknown                                                       | 6.11E-05 | 2.83     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Catabacteriaceae                                              | 0.0022   | 6.79     |
| FAMILY         | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae                                               | 0.0079   | -1.49    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/unknown                                                       | 6.11E-05 | 2.83     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Catabacteriaceae/unknown                                      | 0.0022   | 6.79     |
| GENUS          | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0079   | -1.49    |
| denovo28886    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0005   | -8.33    |
| denovo30978    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0017   | -4.75    |
| denovo14829    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0020   | -14.00   |
| denovo23619    | Firmicutes/Clostridia/Clostridiales                                                               | 0.0032   | Low      |
| denovo721      | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus vaginalis</i> | 0.0063   | High     |
| denovo39924    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0064   | -7.75    |
| denovo14082    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0070   | -3.83    |
| denovo27448    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0086   | -14.00   |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                               | p-value  | FoldGood |
| denovo4280     | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0031   | High     |
| denovo31256    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                      | 0.0046   | -7.00    |
| denovo24938    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                      | 0.0071   | High     |
| denovo22297    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                               | 0.0088   | 2.52     |

**Table S2** | Pearson correlations between different levels of taxa and **FCR** values in all birds from each trail.

|                |                                                                    |         |       |
|----------------|--------------------------------------------------------------------|---------|-------|
| <b>TRIAL 1</b> | GreenGenes taxonomy                                                | p-value | r     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae                | 0.0004  | -0.43 |
| FAMILY         | Tenericutes/Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae | 0.0053  | -0.34 |

|                |                                                                                                               |                |          |
|----------------|---------------------------------------------------------------------------------------------------------------|----------------|----------|
| FAMILY         | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae                                                        | 0.0181         | 0.29     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                           | 0.0260         | -0.27    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Clostridium</i>                                       | 0.0004         | -0.42    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus</i>                                      | 0.0094         | -0.32    |
| GENUS          | Tenericutes/Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/ <i>Clostridium</i>                        | 0.0118         | -0.31    |
| GENUS          | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides</i>                                    | 0.0181         | 0.29     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                                  | 0.0217         | -0.28    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/unknown                                                   | 0.0364         | -0.26    |
| denovo10379    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                           | 0.0005         | -0.42    |
| denovo33809    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>               | 0.0008         | 0.40     |
| denovo22831    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>               | 0.0020         | 0.37     |
| denovo29625    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>               | 0.0030         | 0.36     |
| denovo37095    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>               | 0.0037         | 0.35     |
| denovo14765    | Tenericutes/Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/ <i>Clostridium/Clostridium spiroforme</i> | 0.0057         | -0.34    |
| denovo37572    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                                  | 0.0092         | -0.32    |
| <b>TRIAL 2</b> | <b>GreenGenes taxonomy</b>                                                                                    | <b>p-value</b> | <b>r</b> |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/unknown                                                                   | 2.54E-05       | -0.47    |
| FAMILY         | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae                                                           | 0.0014         | 0.37     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Catabacteriaceae                                                          | 0.0016         | -0.36    |
| FAMILY         | Tenericutes/Mollicutes/RF39/unknown                                                                           | 0.0212         | -0.27    |
| FAMILY         | Proteobacteria/Alphaproteobacteria/unknown                                                                    | 0.0636         | -0.22    |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae                                                           | 0.0950         | 0.20     |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/ClostridialesFamilyXIII.IncertaeSedis                                     | 0.1022         | -0.19    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/unknown                                                                   | 2.54E-05       | -0.47    |
| GENUS          | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                                     | 0.0014         | 0.37     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Catabacteriaceae/unknown                                                  | 0.0016         | -0.36    |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Coproccoccus</i>                                      | 0.0019         | 0.36     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus</i>                                      | 0.0031         | 0.34     |

|                |                                                                                                   |          |       |
|----------------|---------------------------------------------------------------------------------------------------|----------|-------|
| GENUS          | Tenericutes/Mollicutes/RF39/unknown                                                               | 0.0212   | -0.27 |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Ruminococcus</i>                          | 0.0411   | -0.24 |
| denovo28886    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 2.55E-07 | 0.56  |
| denovo30978    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 1.34E-06 | 0.53  |
| denovo38034    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 1.83E-06 | 0.53  |
| denovo14829    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0002   | 0.43  |
| denovo18242    | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Coproccoccus</i>                          | 0.0009   | 0.38  |
| denovo21177    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0010   | 0.38  |
| denovo12890    | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus/Ruminococcus torques</i>     | 0.0014   | 0.37  |
| denovo26936    | Firmicutes/Clostridia/Clostridiales                                                               | 0.0039   | -0.33 |
| denovo21268    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>   | 0.0041   | 0.33  |
| denovo13055    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                               | 0.0062   | 0.32  |
| denovo2375     | Firmicutes/Clostridia/Clostridiales/Catabacteriaceae_                                             | 0.0072   | -0.31 |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                               | p-value  | r     |
| denovo22917    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0035   | 0.36  |
| denovo12721    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                         | 0.0045   | 0.35  |
| denovo9041     | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i> | 0.0058   | -0.34 |
| denovo17075    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Ruminococcus</i>                          | 0.0075   | 0.33  |
| denovo6563     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Ruminococcus</i>                          | 0.0080   | -0.33 |

**Table S3** | Different levels of taxa significantly (QIIME t-test,  $p < 0.01$ ) differentially abundant between microbial communities sampled from the 12vs. 12 extreme birds based on AME. High indicates that taxa are present only in high AME birds. Positive fold is more abundant in high AME birds.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                                | p-value | FoldGood |
|----------------|----------------------------------------------------------------------------------------------------|---------|----------|
| denovo33326    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0062  | Low      |
| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                                | p-value | FoldGood |
| denovo32578    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0023  | -5.66    |
| denovo5661     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0024  | 16.00    |
| denovo14082    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0036  | -8.00    |
| denovo25651    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                       | 0.0047  | 3.22     |
| denovo28886    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0069  | -4.00    |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                                                | p-value | FoldGood |
| denovo28337    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0062  | High     |
| denovo7979     | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0090  | -9.00    |

**Table S4** | Pearson correlations between different levels of taxa and AME values in all birds from each trail.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                             | p-value | r     |
|----------------|-------------------------------------------------------------------------------------------------|---------|-------|
| denovo29914    | Tenericutes/Mollicutes/RF39                                                                     | 0.0021  | -0.37 |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                                             | p-value | r     |
| denovo28886    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i> | 0.0000  | -0.50 |

|                |                                                                                                   |         |       |
|----------------|---------------------------------------------------------------------------------------------------|---------|-------|
| denovo14082    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0033  | -0.34 |
| denovo32578    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0050  | -0.33 |
| denovo27306    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>   | 0.0092  | -0.30 |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                               | p-value | r     |
| denovo23735    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                               | 0.0049  | -0.35 |
| denovo15229    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i> | 0.0076  | 0.33  |
| denovo8803     | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/Coproccoccus                                  | 0.0081  | 0.33  |

**Table S5** | Different levels of taxa significantly (QIIME t-test,  $p < 0.01$ ) differentially abundant between microbial communities sampled from the 12vs. 12 extreme birds based on GR. High indicate that taxa are present only in high **GR** birds. Positive fold indicates higher abundance in high Growth Rate birds.

|                |                                                                           |         |          |
|----------------|---------------------------------------------------------------------------|---------|----------|
| <b>TRIAL 1</b> | GreenGenes taxonomy                                                       | p-value | FoldGood |
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                       | 0.0082  | 2.37     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/unknown               | 0.0093  | 2.26     |
| denovo8806     | Firmicutes/Clostridia/Clostridiales                                       | 0.0004  | Low      |
| denovo32512    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i> | 0.0023  | Low      |
| denovo10379    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                       | 0.0094  | -3.00    |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                       | p-value | FoldGood |
| denovo4753     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                       | 0.0115  | Low      |

**Table S6** | Pearson correlations between different levels of taxa and **GR** values in all birds from each trail.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                                | p-value  | r     |
|----------------|----------------------------------------------------------------------------------------------------|----------|-------|
| FAMILY         | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0108   | 0.31  |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/unknown                                        | 0.0112   | 0.31  |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                       | 0.0299   | 0.27  |
| denovo10379    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 1.47E-08 | 0.63  |
| denovo37572    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                       | 0.0002   | 0.45  |
| denovo10043    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>    | 0.0003   | 0.43  |
| denovo32512    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0008   | 0.40  |
| denovo8806     | Firmicutes/Clostridia/Clostridiales                                                                | 0.0011   | 0.39  |
| denovo12953    | Tenericutes/Mollicutes/RF39                                                                        | 0.0037   | 0.35  |
| denovo8561     | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0071   | 0.33  |
| denovo6371     | Tenericutes/Mollicutes/RF39                                                                        | 0.0092   | 0.32  |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                                                | p-value  | r     |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus</i>                           | 0.0020   | -0.36 |
| GENUS          | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Clostridium</i>                            | 0.0078   | -0.31 |
| denovo4753     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0001   | -0.44 |
| denovo21177    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0046   | -0.33 |
| denovo12890    | Firmicutes/Clostridia/Clostridiales/Lachnospiraceae/ <i>Ruminococcus/Ruminococcus torques</i>      | 0.0054   | -0.32 |
| denovo38034    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0087   | -0.30 |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                                | p-value  | r     |
| denovo17576    | Firmicutes/Clostridia/Clostridiales/                                                               | 0.0033   | 0.36  |
| denovo32969    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0052   | 0.35  |
| denovo24223    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i>  | 0.0071   | 0.33  |
| denovo31632    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0072   | 0.33  |



|             |                                                                                                   |        |      |
|-------------|---------------------------------------------------------------------------------------------------|--------|------|
| denovo23888 | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i> | 0.0079 | 0.33 |
|-------------|---------------------------------------------------------------------------------------------------|--------|------|

**Table S7** | Different levels of taxa significantly (QIIME t-test,  $p < 0.01$ ) differentially abundant between microbial communities sampled from the 12vs. 12 extreme birds based on FE. High indicates that taxa is present only in high **FE** birds. Positive fold indicates higher abundance in high FE, negative fold indicates higher abundance in low FE.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                                | p-value  | FoldHigh |
|----------------|----------------------------------------------------------------------------------------------------|----------|----------|
| denovo37369    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>    | 0.0076   | -7.50    |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                                                | p-value  | FoldHigh |
| FAMILY         | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae                                             | 0.0053   | -3.10    |
| GENUS          | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides</i>                         | 0.0053   | -3.10    |
| denovo26333    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0100   | High     |
| denovo28895    | Firmicutes/Clostridia/Clostridiales                                                                | 0.0100   | High     |
| denovo27497    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0106   | 3.83     |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                                | p-value  | FoldHigh |
| denovo15167    | Firmicutes/Clostridia/Clostridiales                                                                | 0.003105 | 3.97     |
| denovo27818    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i>  | 0.006492 | Low      |
| denovo12985    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.007045 | 8.71     |
| denovo22961    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae/ <i>Faecalibacterium</i>                       | 0.00931  | -17.45   |

**Table S8** | Pearson correlations between different levels of taxa and **FE** values in all birds from each trail.

| <b>TRIAL 1</b> | GreenGenes taxonomy                                                                                | p-value | r     |
|----------------|----------------------------------------------------------------------------------------------------|---------|-------|
| FAMILY         | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae                                                | 0.0062  | 0.33  |
| GENUS          | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0062  | 0.33  |
| denovo37369    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>    | 0.0016  | -0.38 |
| denovo10043    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>    | 0.0016  | -0.38 |
| denovo11094    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0025  | 0.37  |
| denovo11042    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0035  | 0.35  |
| denovo33813    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus</i>                          | 0.0041  | 0.35  |
| denovo38138    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0087  | 0.32  |
| denovo33326    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus salivarius</i> | 0.0094  | 0.32  |
| <b>TRIAL 2</b> | GreenGenes taxonomy                                                                                | p-value | r     |
| FAMILY         | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae                                             | 0.0015  | -0.36 |
| GENUS          | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides</i>                         | 0.0015  | -0.36 |
| GENUS          | Tenericutes/Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/ <i>Clostridium</i>             | 0.0032  | -0.34 |
| denovo4753     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0005  | -0.40 |
| denovo7572     | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0007  | -0.39 |
| denovo15209    | Bacteroidetes/Bacteroidia/Bacteroidales/Bacteroidaceae/ <i>Bacteroides/Bacteroides fragilis</i>    | 0.0019  | -0.36 |
| denovo13545    | Firmicutes/Clostridia/Clostridiales/Ruminococcaceae                                                | 0.0023  | -0.35 |
| denovo27999    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i>  | 0.0077  | 0.31  |
| denovo27497    | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus reuteri</i>    | 0.0094  | 0.30  |
| <b>TRIAL 3</b> | GreenGenes taxonomy                                                                                | p-value | r     |
| denovo7211     | Firmicutes/Bacilli/Lactobacillales/Lactobacillaceae/ <i>Lactobacillus/Lactobacillus crispatus</i>  | 0.0048  | -0.35 |
| denovo32968    | Tenericutes/Mollicutes/RF39                                                                        | 0.0057  | -0.34 |

## References

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