**Supplementary Material**

**Taxonomic and functional assessment reveals the effect of Angus breed genetics on rumen microbial signatures**

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Short title: Active Rumen Microbes and Microbial Functions in Beef Cattle

**Supplementary Tables**

Supplementary Table S1. Chemical composition of the experimental diet1

|  |  |
| --- | --- |
| Item | Forage |
| **Ingredient composition, %, as-fed basis** |   |
|  Alfalfa Hay | 18.0 |
|  Corn Silage | 81.5 |
|  Limestone | 0.3 |
|  Mineral | 0.1 |
|  Salt | 0.1 |
| **Chemical composition, DM basis** |   |
|  Dry Matter, % | 50.7 |
|  Acid Detergent Fiber, % | 21.78 |
|  Neutral Detergent Fiber, % | 40.89 |
|  Total Digestible Nutrients, % | 73.51 |
|  Starch, % | 21.6 |
|  Metabolizable Energy, MJ kg-1 | 11.10 |
|  Crude Protein, % | 13.1 |
|  Calcium, %  | 0.89 |
|  Phosphorus, % | 0.45 |
|  Magnesium, % | 0.42 |
|  Potassium, % | 2.16 |

1The chemical analysis of the feed was conducted in the Department of Animal Science & National Centre for Livestock and the Environment (NCLE), University of Manitoba, Winnipeg, MB, Canada.

**Supplementary Tables S2, S3 and S4**

Here we present a complete list of bacteria classified by Kraken (Supplementary Table S2) and microbial gene families detected by ShotMAP (Supplementary Table S3) in all bulls. We also present a direct comparison of bacteria and gene families characterizing the rumen microbiome of Black and Red Angus cattle according to the sPLS-DA results (Supplementary Table S4).

In Supplementary Tables S2 and S3, the results (presented in descending order based on the relative abundance) are shown in each dataset as follows: a) Taxa and microbial functions are in Column 1; and b) IDs of rumen samples collected from Black and Red Angus are shown from Column 2 to Column 25.

In Supplementary Table S4, the results (based on the counts) are shown for each bacterial species (Components 1 and 2) and microbial gene family (Component 1) selected by the sPLS-DA multivariate regression models to discriminate and characterize the rumen microbiota of Black and Red Angus cattle.

Files:

Supplementary Table S2\_Bacteria.xlsx

Supplementary Table S3\_GeneFamilies.xlsx

Supplementary Table S4\_sPLS\_DA.xlsx

# Supplementary Figures



**Figure S1.** Classification performance per component for three predictions distances using repeated stratified cross-validation (10 × 5-fold Cross Validation). In this study, the distance metrics used for sPLS-DA (CLR transformed data) to estimate the classification error rate for the bacterial dataset was the “centroids.dist”.



**Figure S2.** Classification performance per component for two predictions distances using repeated stratified cross-validation (10 × 5-fold Cross Validation). In this study, the distance metrics used for sPLS-DA (CLR transformed data) to estimate the classification error rate for the microbial functions dataset was the “centroids.dist”.



**Figure S3.** Classification performance per component for two predictions distances using repeated stratified cross-validation (10 × 5-fold Cross Validation). The distance metrics used for sPLS-DA (Relative Abundance data) to estimate the classification error rate for the bacterial dataset was the “centroids.dist”.



**Figure S4.** Contribution plot of each species selected on the first component (Relative Abundance data). Length of the bar represents the importance of each species to the component (from bottom to top). Colors indicate the breed (Black vs. Red Angus) in which the species is most abundant.

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**Figure S5.** Power analysis for between-effect tests to detect FCR differences between Black and Red Angus. The figure shows that the minimal sample size to detect a FCR effect size of 1.5, with a power of 0.9 and p-value <0.5 in samples collected from Black and Red Angus over four-time points is of 9 animals per breed.