**A rapid shift to high-grain diet results in dynamic changes in rumen epimural microbiome in sheep**

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***Animal* journal Supplementary Materials**

**Supplementary Material S1**

**S1. Materials and methods**

*S.1.1. Rumen fermentation parameters*

Ruminal pH was determined instantly by a pH meter (PB-10; Sartorius, Goettingen, Germany) in a homogenized sample of rumen fluid. Rumen fluid samples were then filtered through four layers of cheesecloth. A sample of the ruminal fluid was kept at –20°C for further analysis of acetate, propionate, butyrate, valerate, isovalerate and isobutyrate using a capillary column gas chromatography (GC-14B; Shimadzu, Japan; Capillary Column: 30 m×0.32 mm×0.25 mm film thickness; Column temperature = 130°C, injector temperature = 180°C, detector temperature = 180°C) (Qin, 1982) and lactate analysis (Barker and Summerson, 1941). The rumen epithelium was rinsed using a physiological solution (NaCl 0.8%). The ruminal epithelial samples were scraped using sterile slides, and then stored at -80°C for subsequent DNA extraction.

*S1.2 Isolation of microbial genomic deoxyribonucleic acid*

The whole genomic *deoxyribonucleic acid* DNA was extracted by applying the procedures of QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). Samples were homogenized by vortex for 1 minute after adding 1 ml of InhibitEX buffer to each sample. Heating for 5 minutes at 70˚C was applied to the suspension before increasing the temperature to 95˚C and vortex for 15 seconds. Centrifugation was applied to pellet particles and the supernatant was transferred into a new tube. 15 µl of Proteinase K and 200µl of buffer AL was added to the supernatant and then incubated at 70˚C for 10 minutes. The resulted lysate was put into a spin column followed by adding 200µl of ethanol, 500 µl of buffer AW1 and 500 µl of buffer AW2, respectively with being filtered after each treatment. AL, AW1 and AW2 are the official names of buffers named by the kit’s manufacturer. 200 µl of elution buffer was added before incubation for 1 min at room temperature and then centrifugation was done to elute DNA. Measuring of DNA was performed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Ultimately, DNA samples were stored at -80˚C for subsequent processing.

*S1.3. 16S ribosomal ribonucleic acid genes amplification and* *high through-put sequencing*

V3-V4 region of bacterial 16S ribosomal ribonucleic acid (rRNA) gene was amplified by (5’-barcode-ACTCCTRCGGGAGGCAGCAG-3’) and (5’-GGACTACCVGGGTATCTAAT-3’) primers (amplicone length 420bp).

Polymerase chain reaction (PCR) products were separated by 2% agarose gel and then purified by AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Quantification of amplicons was performed using QuantiFluorTM – ST (Promega, Durham, NC, USA). Ilumina TruSeq DNA sample preparation Kit (Illumina, San Diego, CA, USA) was used to construct a sequencing library. Cluster generation, isothermal amplification, template hybridization, linearization, hybridization, blocking and denaturisation was performed using Illumina TruSeq PE Cluster and Sequencing by Synthesis (SBS) Kits. Paired-end sequencing 2×300 base pair was done to sequence all libraries on Illumina MiSeq platform (Caporaso *et al.*, 2012).

QIIME software package (version 1.70) (<http://qiime.org/>) (Campbell *et al.*, 2010) was used to analyze the sequence data. Sequence reads were assigned to each sample before being trimmed based on their barcodes. High quality sequences (>250 bp without ambiguous base ‘N’ and average base quality score >25) were used for downstream analysis. UCHIME (<http://drive5.com/usearch/manual/uchime_algo.html>) was used to filter chimeric sequences (Edgar, 2010) and then sequences were clustered into operational taxonomic units (OTUs) de novo based on similarity 97% using UPARSE (<http://drive5.com/uparse/>). RDP classifier (Release 11.1 <http://rdp.cme.msu.edu/>) was used to assign representative sequences from each OTU (Wang *et al.*, 2007). The default parameters set by QIIME were maintained while aligning these representative sequences to the SILVA reference database [(Release119 http://www.arb-silva.de);](file:///C:\Users\Hossam\Downloads\(Release119%20http:\www.arb-silva.de);) by using PyNAST (Caporaso *et al.*, 2010). Diversity of community was estimated using the ACE1, Chao1, Shannon and simpson indices. Phylogenetic tree of the representative sequences was created by FASTTREE (<http://www.microbesonline.org/fasttree/>) (Price *et al.*, 2009). Principle coordinate analysis was performed by unweighted UniFrac distance method (Lozupone and Knight, 2005). Significant differences among samples were tested by unweighted distance-based analysis of molecular variance (AMOVA) using MOTHUR program (<https://www.mothur.org/>) (version 1.29) (Schloss *et al.*, 2009).

*S1.4. Predicted metabolic capacity analysis*

In the present study, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) tool to predict the metabolic functions of each sample based on 16S rRNA data. PICRUSt is a bioinformatics tool that depends on marker genes, 16S rRNA in our study, to predict the genomic functional capabilities of microorganisms. In the present study, we used the KEGG database and did closed reference OTU picking based on the sampled reads against Greengenes database (Greengenes 13.5) (release 13.5) (http://greengenes.secondgenome.com/). Genomic metabolic functions were predicted using the predict\_metagenomes.py and data were summarized into Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (<http://www.genome.jp/kegg/>) using the categorize\_by\_function.py script, all included in PICRUSt <http://picrust.github.io/picrust/> (Langille *et al.*, 2013)*.*

*S1.5. Statistical analysis*

Statistical calculations were carried out by conducting tests using the SPSS software package (SPSS version 23, SPSS, Inc.). The fermentation data and pH value obtained were analyzed using one-way ANOVA. The hypotheses were tested using contrast test (treatment: CON vs. HG) to evaluate the diet effect and polynomial contrasts (linear, quadratic, and cubic effects) to evaluate whether time of HG feeding resulted in linear, quadratic, or cubic patterns accounting for unequal durations that sheep were fed the HG diet. For the relative abundance of the phyla, genera and KEGGs, The R statistics npmv (Burchett *et al.*, 2017) package (<https://www.r-project.org>) was used to deduce the global variance in the relative abundance of the phyla, genera and KEGGs.

The Kruskal–Wallis test was used to test the variance between groups across each phylum, genus and KEGG family, followed by pair-wise comparison between groups’ means. Correlations between variables were tested by Pearson correlation test using GraphPad Prism version 6.00 (<https://www.graphpad.com/scientific-software/prism/>) (GraphPad Software). Significance was set at 0.05.

**S2. Results**

*S2.1. Results of quality control procedures*

Number of sequences and average sequence length after trimming and chimera checking are shown in (Supplementary Table S7). It can be noticed that the average number of sequences in each sample was 43326 and the average length was 416. Rarefaction curves obtained for each sample are shown in Supplementary Figure S1. It can be noticed that rarefaction curves of each sample have reached a plateau, suggesting an adequate sequence depth for detecting the majority of the bacteria present in the samples. For the PICRUSt analysis, results showed that 72.5 % of total OTUs were able to be mapped to known genomes. The ratio of mapped OTUs of each phylum is also illustrated in Supplementary Table S8.

**S4. References**

*S4.1. References cited in the supplementary materials*

Burchett WW, Ellis AR, Harrar SW and Bathke AC 2017. Nonparametric Inference for Multivariate Data: The R Package npmv. Journal of Statistical Software 76, 1-18.

Campbell BJ, Polson SW, Hanson TE, Mack MC and Schuur EAG 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12, 1842-1854.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL and Knight R 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266-267.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G and Knight R 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6, 1621-1624

Edgar RC 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-2461.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG and Huttenhower C 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31, 814.

Lozupone C and Knight R 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71, 8228-8235.

Price MN, Dehal PS and Arkin AP 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Molecular Biology and Evolution 26, 1641-1650.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH and Robinson CJ 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537-7541.

Wang Q, Garrity GM, Tiedje JM and Cole JR 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261.

*S4.2. References cited in the manuscript*

Russell J, Garner M and Flint J 2002. Allisonella histiformans, sp. nov., a novel bacterium that produces histamine, utilizes histidine as its sole energy source, and could play a role in bovine and equine laminitis. Systematic and Applied Microbiology 25, 498-506.

Stewart D 1977. Biochemical and biological studies on the lipopolysaccharide of Bacteroides nodosus. Research in Veterinary Science 23, 319-325.

Ye H, Liu J, Feng P, Zhu W and Mao S 2016. Grain-rich diets altered the colonic fermentation and mucosa-associated bacterial communities and induced mucosal injuries in goats. Scientific Reports 6, 20329.

Ze X, Duncan SH, Louis P and Flint HJ 2012. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. The ISME Journal 6, 1535-1543.

Zhao L, Meng Q, Ren L, Liu W, Zhang X, Huo Y and Zhou Z 2015. Effects of nitrate addition on rumen fermentation, bacterial biodiversity and abundance. Asian-Australasian Journal of Animal Sciences 28, 1433.

Zhao S, Wang J and Bu D 2014. Pyrosequencing-based profiling of bacterial 16S rRNA genes identifies the unique Proteobacteria attached to the rumen epithelium of bovines. Journal of Dairy Science 97, 869-870

**Table S1** *Experimental diets ingredients and proximate analysis of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

|  |  |  |
| --- | --- | --- |
| Item | Diet | |
| Control | High-grain |
| Ingredient composition, DM % |  |  |
| Oat hay | 63.40 | 26.00 |
| Alfalfa hay | 33.00 | 14.00 |
| Corn meal | 0 | 34.20 |
| Wheat meal | 0 | 18.00 |
| Soybean meal | 0 | 4.20 |
| CaCO3 | 1.00 | 1.00 |
| NaCl, salt | 0.40 | 0.40 |
| CaHPO4 | 1.20 | 1.20 |
| Mineral and vitamin supplement | 1.00 | 1.00 |
| Nutrient composition |  |  |
| DE, MJ/kg DM | 8.88 | 11.73 |
| Crude protein, DM | 11.18 | 11.92 |
| Crude fat, DM | 2.09 | 2.49 |
| Crude fiber, DM | 28.41 | 12.90 |
| Neutral detergent fiber, DM | 44.45 | 24.54 |
| Acid detergent fiber, DM | 19.52 | 10.15 |
| Crude ash, DM | 8.34 | 4.53 |
| Starch, DM | 3.25 | 32.34 |

DM, Dry matter based

DE, Digestible energy

**Table S2.** *AMOVA of unweighted PCoA of rumen epimural bacteriome in sheep fed hay (CON) or abruptly shifted to a high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | CON | HG7 | HG14 | HG28 |
| CON |  |  |  |  |
| HG7 | 0.002 |  |  |  |
| HG14 | 0.001 | 0.058 |  |  |
| HG28 | 0.003 | 0.034 | 0.062 |  |

**Table S3.** *The global variance test results of nonparametric multivariate data using npmv R package for the relative abundance of phyla, Genera, OTUs and KEGGs of rumen epimural microbiome of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

*(Burchett, 2017)*

|  |  |  |
| --- | --- | --- |
|  | Permutation *P* | |
| Diet1 | Adaptation2 |
| Phyla | <0.001 | 0.11 |
| Genera | <0.001 | 0.001 |
| OTUs | <0.001 | 0.011 |
| KEGGs | 0.001 | 0.545 |

1Control group was included in the test

2Control group was not included in the test

OTU = Operational Taxonomic Unit

KEGG = Kyoto Encyclopedia of Genes and Genomes

**Table S4** *The relative abundance of bacterial phyla in rumen epimural microbiome of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Relative abundance (%) | | | | SEM | *P-value* | |
| CON | HG7 | HG14 | HG28 | Diet1 | Adaptation2 |
| Bacteroidetes | 34.25 | 41.14 | 38.67 | 33.04 | 1.68 | 0.226 | 0.326 |
| Firmicutes | 36.80 | 39.90 | 41.83 | 47.67 | 1.96 | 0.187 | 0.230 |
| Proteobacteria | 22.09a | 11.38b | 10.42b | 11.51b | 1.321 | 0.042 | 0.914 |
| Fibrobacteres | 1.59 | 1.30 | 0.79 | 0.75 | 0.19 | 0.177 | 0.402 |
| Spirochaetes | 2.85b | 4.60b | 7.14a | 3.48b | 0.6 | 0.046 | 0.048 |
| Cyanobacteria | 0.36a | 0.29ab | 0.06b | 0.09ab | 0.05 | 0.029 | 0.141 |
| Candidate\_division\_TM7 | 0.31 | 0.20 | 0.14 | 0.15 | 0.04 | 0.514 | 0.932 |
| Synergistetes | 0.38 | 0.31 | 0.39 | 0.33 | 0.05 | 0.870 | 1.000 |
| Actinobacteria | 0.50 | 0.62 | 0.30 | 2.60 | 0.54 | 0.295 | 0.174 |
| Elusimicrobia | 0.13 | 0.07 | 0.03 | 0.03 | 0.02 | 0.227 | 0.365 |
| Candidate\_division\_SR1 | 0.37a | 0.01b | 0.05ab | 0.19ab | 0.04 | 0.010 | 0.135 |
| Tenericutes | 0.22 | 0.14 | 0.12 | 0.09 | 0.02 | 0.071 | 0.578 |
| Lentisphaerae | 0.08a | 0.02ab | 0.01b | 0.03ab | 0.01 | 0.030 | 0.508 |
| Chloroflexi | 0.02 | 0.01 | 0.01 | 0.01 | <0.01 | 0.761 | 0.523 |
| Verrucomicrobia | 0.02a | 0.01ab | <0.00b | <0.00ab | <0.01 | 0.030 | 0.581 |
| Chlorobi | 0.01a | <0.00b | <0.00b | <0.00b | <0.01 | 0.003 | 1.000 |
| SHA-109 | 0.01 | - | - | <0.01 | <0.01 | 0.051 | 0.368 |
| BD1-5 | <0.01 | - | - | <0.00 | <0.01 | 0.409 | 0.581 |
| Deinococcus-Thermus | <0.01 | - | <0.00 | - | <0.01 | 0.519 | 0.362 |
| Fusobacteria | <0.01 | - | 0.04 | 0.01 | <0.01 | 0.244 | 0.161 |

1Control group is included in the test

2Control group is not included in the test

a,b Mean values within a row with different superscript lower case letters are significantly different (*P* ≤ 0.05) in the analysis that comprised all the groups.

(-) not detected

**Table S5**. The operational taxonomic units (OTUs) that showed a significant difference due to the duration of the high-grain diet feeding *in rumen epimural microbiome of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| OTU ID | Relative abundance (%) | | | SEM | *P value*1 | Phylum | Genus | Highest available identity |
| HG7 | HG14 | HG28 |
| OTU1 | 3.99 | 1.73 | 1.95 | 0.90 | 0.013 | Proteobacteria | *Campylobacter* | Uncultured rumen bacterium |
| OTU33 | 0.52 | 1.36 | 0.61 | 0.13 | 0.040 | Proteobacteria | *Desulfobulbus* | Uncultured rumen bacterium |
| OTU231 | 0.01 | <0.01 | 0.25 | 0.04 | 0.020 | Firmicutes | Unclassified Lacnospiraceae | Uncultured rumen bacterium |
| OTU98 | 0.04 | 0.53 | 0.14 | 0.07 | 0.043 | Bacteroidetes | RC9\_gut\_group | Uncultured rumen bacterium |
| OTU128 | 0.02 | 0.10 | 0.38 | 0.07 | 0.024 | Firmicutes | *Selenomonas* | Uncultured rumen bacterium |
| OTU127 | 0.19 | <0.01 | 0.30 | 0.07 | 0.015 | Actinobacteria | *Bifidobacterium* | *Bifidobacterium pseudolongum* |
| OTU101 | 0.02 | 0.03 | 0.56 | 0.12 | 0.049 | Bacteroidetes | Unclassified BS11 gut group | Uncultured rumen bacterium |
| OTU51 | 0.68 | 0.06 | 0.22 | 0.07 | 0.007 | Firmicutes | *Howardella* | Uncultured rumen bacterium |
| OTU136 | 0.10 | 0.05 | 0.23 | 0.03 | 0.019 | Firmicutes | *Butyrivibrio* | Uncultured rumen bacterium |
| OTU93 | 0.64 | <0.01 | 0.02 | 0.12 | 0.045 | Firmicutes | *Ruminococcus* | Uncultured rumen bacterium |
| OTU83 | 0.53 | 0.15 | 0.11 | 0.06 | 0.034 | Firmicutes | Unclassified family XIII | Uncultured rumen bacterium |
| OTU20 | 0.90 | 1.55 | 0.41 | 0.24 | 0.022 | Bacteroidetes | *Prevotella* | Uncultured rumen bacterium |
| OTU120 | 0.35 | 0.08 | 0.02 | 0.05 | 0.019 | Bacteroidetes | *Prevotella* | Uncultured rumen bacterium |
| OTU220 | 0.01 | 0.03 | 0.17 | 0.03 | 0.018 | Actinobacteria | *Atopobium* | Uncultured rumen bacterium |
| OTU105 | 0.47 | 0.04 | 0.14 | 0.07 | 0.042 | Firmicutes | *Ruminococcus* | Uncultured rumen bacterium |
| OTU77 | 0.49 | 0.10 | 0.37 | 0.05 | 0.030 | Firmicutes | *Syntrophococcus* | Uncultured rumen bacterium |
| OTU39 | 0.93 | 0.47 | 0.06 | 0.16 | 0.026 | Fibrobacteres | *Fibrobacter* | Uncultured rumen bacterium |
| OTU92 | 0.14 | 0.02 | 0.71 | 0.12 | 0.026 | Bacteroidetes | *Prevotella* | Uncultured rumen bacterium |
| OTU32 | 2.02 | 0.42 | 0.00 | 0.34 | 0.024 | Bacteroidetes | Unclassified prevotellaceae | Uncultured rumen bacterium |
| OTU111 | 0.72 | 0.00 | 0.00 | 0.16 | 0.031 | Bacteroidetes | *Prevotella* | Uncultured rumen bacterium |
| OTU121 | 0.12 | 0.00 | 0.00 | 0.05 | 0.001 | Fibrobacteres | *Fibrobacter* | Uncultured rumen bacterium |

1 The Kruskal-Wallis test was done among the 3 HG groups only.

**Table S6** *The relative abundance of KEGG genes in rumen epimural microbiome of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Relative abundance (%) | | | | SEM | *P-*value | |
| CON | HG7 | HG14 | HG28 | Diet1 | Adaptation2 |
| Cellular processes |  |  |  |  |  |  |  |
| Cell Motility | 3.24 | 3.22 | 3.65 | 3.37 | 0.09 | 0.265 | 0.203 |
| Cell Growth and Death | 0.54 | 0.53 | 0.53 | 0.52 | 0.00 | 0.341 | 0.882 |
| Transport and Catabolism | 0.28 | 0.26 | 0.26 | 0.24 | 0.01 | 0.153 | 0.553 |
| Environmental Information Processing |  |  |  |  |  |  |  |
| Membrane Transport | 11.52 | 11.38 | 11.27 | 11.78 | 0.17 | 0.807 | 0.651 |
| Signal Transduction | 1.73 | 1.70 | 1.83 | 1.71 | 0.03 | 0.296 | 0.173 |
| Signaling Molecules and Interaction | 0.14 | 0.15 | 0.15 | 0.15 | 0.00 | 0.561 | 0.902 |
| Genetic Information Processing |  |  |  |  |  |  |  |
| Replication and Repair | 9.08 | 8.88 | 8.80 | 8.95 | 0.05 | 0.204 | 0.405 |
| Translation | 6.05 | 6.06 | 6.02 | 6.02 | 0.05 | 0.994 | 0.990 |
| Folding, Sorting and Degradation | 2.57 | 2.48 | 2.48 | 2.46 | 0.02 | 0.128 | 0.906 |
| Transcription | 2.50 | 2.60 | 2.54 | 2.64 | 0.02 | 0.105 | 0.343 |
| Human diseases |  |  |  |  |  |  |  |
| Infectious Diseases | 0.35 | 0.37 | 0.38 | 0.38 | 0.01 | 0.061 | 0.821 |
| Neurodegenerative Diseases | 0.24 a | 0.15 b | 0.14 b | 0.13 b | 0.01 | 0.005 | 0.213 |
| Cancers | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 | 0.494 | 0.350 |
| Metabolic Diseases | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 | 0.761 | 0.664 |
| Immune System Diseases | 0.04 | 0.05 | 0.05 | 0.05 | 0.00 | 0.186 | 0.584 |
| Metabolism |  |  |  |  |  |  |  |
| Amino Acid Metabolism | 9.98 | 9.94 | 9.76 | 9.80 | 0.04 | 0.128 | 0.221 |
| Carbohydrate Metabolism | 9.43 b | 9.92 a | 9.85 a | 9.95 a | 0.07 | 0.027 | 0.757 |
| Energy Metabolism | 6.04 | 6.18 | 6.34 | 6.11 | 0.06 | 0.36 | 0.357 |
| Metabolism of Cofactors and Vitamins | 4.28 | 4.25 | 4.26 | 4.30 | 0.04 | 0.941 | 0.914 |
| Nucleotide Metabolism | 4.11 | 4.22 | 4.15 | 4.17 | 0.03 | 0.539 | 0.852 |
| Lipid Metabolism | 2.85 a | 2.73 ab | 2.64 b | 2.63 b | 0.03 | 0.012 | 0.190 |
| Glycan Biosynthesis and Metabolism | 2.42 | 2.20 | 2.15 | 2.13 | 0.05 | 0.089 | 0.590 |
| Enzyme Families | 2.09 | 2.14 | 2.13 | 2.15 | 0.01 | 0.151 | 0.790 |
| Xenobiotics Biodegradation and Metabolism | 1.84 | 1.80 | 1.77 | 1.67 | 0.02 | 0.062 | 0.088 |
| Metabolism of Terpenoids and Polyketides | 1.77 a | 1.71 b | 1.71 b | 1.68 b | 0.01 | 0.032 | 0.322 |
| Metabolism of Other Amino Acids | 1.54 a | 1.44 b | 1.42 b | 1.47 ab | 0.01 | 0.018 | 0.394 |
| Biosynthesis of Other Secondary Metabolites | 0.87 | 0.91 | 0.91 | 0.92 | 0.01 | 0.233 | 0.982 |
| Organismal systems |  |  |  |  |  |  |  |
| Endocrine System | 0.28 | 0.29 | 0.27 | 0.27 | 0.00 | 0.436 | 0.311 |
| Environmental Adaptation | 0.17 | 0.17 | 0.18 | 0.18 | 0.00 | 0.244 | 0.554 |
| Nervous System | 0.09 | 0.10 | 0.09 | 0.09 | 0.00 | 0.06 | 0.108 |
| Immune System | 0.08 | 0.08 | 0.08 | 0.08 | 0.00 | 0.287 | 0.308 |
| Circulatory System | 0.04a | 0.02b | 0.01 b | 0.01 b | 0.00 | 0.004 | 0.136 |
| Digestive System | 0.03 | 0.04 | 0.04 | 0.04 | 0.00 | 0.087 | 0.591 |
| Excretory System | 0.02 a | 0.02 a | 0.02 a | 0.01 b | 0.00 | 0.043 | 0.097 |
| Unclassified |  |  |  |  |  |  |  |
| Poorly Characterized | 4.83 b | 5.12 a | 5.20 a | 5.01a | 0.05 | 0.007 | 0.300 |
| Cellular Processes and Signaling | 3.7 a | 3.51b | 3.46 b | 3.55b | 0.04 | 0.027 | 0.608 |
| Genetic Information Processing | 2.72 b | 2.83 ab | 2.89 a | 2.79ab | 0.02 | 0.026 | 0.300 |
| Metabolism | 2.32 | 2.35 | 2.40 | 2.38 | 0.01 | 0.069 | 0.186 |

1Control group was included in the test

2Control group was not included in the test

KEGG = Kyoto Encyclopedia of Genes and Genomes

a,b Mean values within a row with dissimilar superscript lower case letters are significantly different (*P* ≤ 0.05) in the analysis that comprised all the groups.

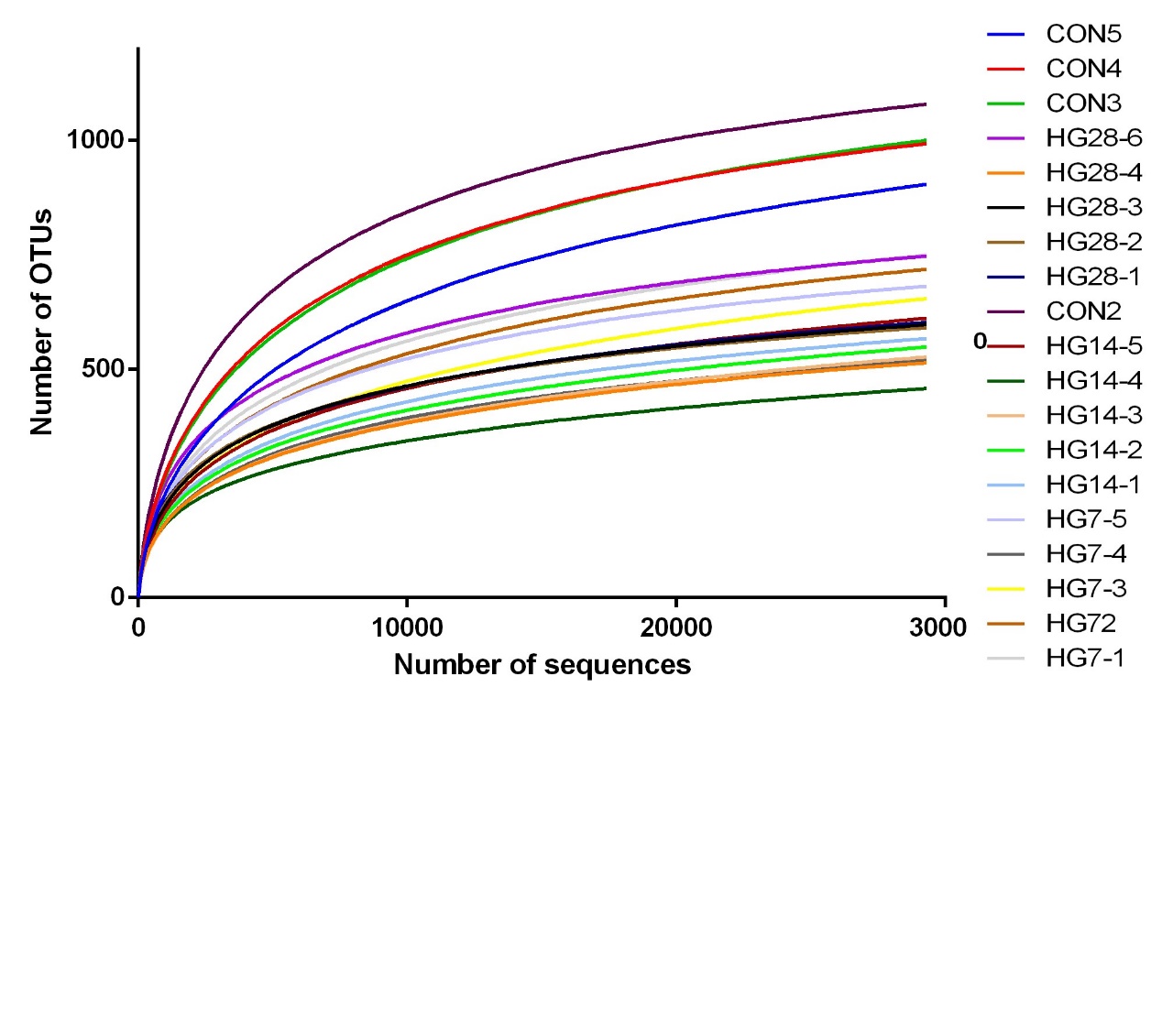
**Table S7** *Number of sequences and average length in each sample of rumen epimural bacteria of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days.*

|  |  |  |
| --- | --- | --- |
| Sample | No. of sequences | Average length (bp) |
| CON-1 | 43256 | 416.28 |
| CON-2 | 38262 | 416.68 |
| CON-3 | 44317 | 416.27 |
| CON-4 | 42378 | 416.07 |
| CON-5 | 44256 | 418.47 |
| HG7-1 | 44593 | 417.69 |
| HG7-2 | 34345 | 415.89 |
| HG7-3 | 37747 | 415.23 |
| HG7-4 | 43291 | 418.52 |
| HG7-5 | 43326 | 416.17 |
| HG14-1 | 44264 | 413.33 |
| HG14-2 | 38826 | 416.98 |
| HG14-3 | 33902 | 419.34 |
| HG14-4 | 31078 | 419.23 |
| HG14-5 | 38603 | 419.22 |
| HG28-1 | 38632 | 415.84 |
| HG28-2 | 32493 | 416.02 |
| HG28-3 | 32042 | 418.49 |
| HG28-4 | 44537 | 414.32 |
| HG28-5 | 33671 | 415.63 |
| Average | 43326 | 416.17 |

**Table S8**. *Proportion of OTUs that could be mapped in the PICRUSt analysis for each phylum of rumen epimural bacteria of sheep fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days.*

|  |  |  |  |
| --- | --- | --- | --- |
| Phylum | Total number of OTUs | No. of OTUs mapped in PICRUSt analysis | % of OTUs mapped in PICRUSt |
| Actinobacteria | 67 | 36 | 53.73% |
| Bacteroidetes | 580 | 382 | 65.86% |
| BD1-5 | 1 | Not mapped | 0.00% |
| Candidate division SR1 | 2 | 2 | 100.00% |
| Candidate division TM7 | 22 | 18 | 81.82% |
| Chlorobi | 1 | Not mapped | 0.00% |
| Chloroflexi | 4 | 3 | 75.00% |
| Cyanobacteria | 41 | 34 | 82.93% |
| Deinococcus-Thermus | 4 | 4 | 100.00% |
| Elusimicrobia | 7 | 6 | 85.71% |
| Fibrobacteres | 19 | 17 | 89.47% |
| Firmicutes | 1272 | 1000 | 78.62% |
| Fusobacteria | 4 | 4 | 100.00% |
| Lentisphaerae | 37 | 21 | 56.76% |
| Proteobacteria | 132 | 88 | 66.67% |
| SHA-109 | 2 | 2 | 100.00% |
| Spirochaetes | 74 | 37 | 50.00% |
| Synergistetes | 8 | 7 | 87.50% |
| Tenericutes | 80 | 47 | 58.75% |
| Verrucomicrobia | 3 | 3 | 100.00% |
| Total | 2360 | 1711 | 72.50% |

OTU = Operational Taxonomic Unit



**Figure S1** *Rarefaction curves obtained based on assigned operational taxonomic units (OTUs) of rumen epimural bacteria of sheep* *fed hay (CON) or abruptly shifted to high-grain diet for 7 (HG7), 14 (HG14) or 28 (HG28) days.*