Additional file 1: Figure S1



Additional file 1: Figure S2



Additional file 2: Table S1

|  |  |  |  |
| --- | --- | --- | --- |
| Feed composition | Content (%) | Nutrient level | Content （%） |
| Ingredient |  | DM | 90.57 |
| Corn | 34 | GE/ (MJ/kg) | 16.6 |
| Barley straw | 30.5 | CP | 14,69 |
| Sunflower cake | 10 | Ca | 1.02 |
| Corn fiber | 4 | Total P | 0.42 |
| Cottonseed meal | 7.4 | CF | 14.78 |
| Wheat middlings | 4 | EE | 1.48 |
| Malt sprouts | 3.5 | Ash | 9.56 |
| Soybean meal | 2 |  |  |
| Limestone | 1 |  |  |
| Molasses | 1.5 |  |  |
| Urea | 0.5 |  |  |
| NaHCO3 | 0.5 |  |  |
| Salt | 0.5 |  |  |
| Mineral premix | 0.2 |  |  |
| CaH2PO4 | 0.32 |  |  |
| Vitamin premix | 0.03 |  |  |
| Monensin (20%) | 0.02 |  |  |
| Sweetening agent | 0.02 |  |  |
| Flavouring agent | 0.01 |  |  |
| Total | 100 |  |  |

Additional file 2: Table S2

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Diversity | C | LT | LW | MW | HW | SEM | *P-value* |
| sobs | 163.25 | 166.5 | 161.25 | 157.75 | 167.25 | 2.37 | 0.747 |
| shannon | 2.81 | 2.68 | 2.71 | 3.08 | 3.31 | 0.12 | 0.411 |
| simpson | 0.19 | 0.25 | 0.22 | 0.12 | 0.1 | 0.03 | 0.288 |
| ace | 179.3 | 183.05 | 173.03 | 168.3 | 178.44 | 2.48 | 0.387 |
| chao | 183.39 | 202.02 | 174.39 | 171.54 | 179.7 | 4.46 | 0.216 |
| coverage | 0.999 | 0.999 | 0.999 | 1 | 1 | 0 | 0.026 |

Additional file 2: Table S3

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Standard Curve | Linear correlation coefficient | Amplification efficiency (E%) |
|
| *P. ruminicola* | Y=-3.2191x + 38.254 | 0.993 | 104.48 |
|
| *F.succinogenes* | Y=-3.4681x + 42.9 | 0.979 | 94.24 |
|
| *S.ruminantium* | Y=-3.46x + 42.461 | 0.992 | 94.54 |
|
| *S.dextrinisolvens* | Y=-3.6137x + 44.128 | 0.996 | 89.11 |
| *V. parvula* | Y=-3.7646x + 44.365 | 0.999 | 84.35 |
|
| Fungi | Y = -3.102x + 37.204 | 0.98 | 110.07 |
|
| Total bacterial | Y = -2.8477x + 35.174 | 0.974 | 124.47 |
|

Additional file 2: Table S4

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| log10 (copies/10 ng) | | | | | | | |
|  | Cold and wind chill | | | | | SEM | *P* values |
| C | LT | LW | MW | HW |
| *P. ruminicola* | 6.164 | 5.485 | 6.182 | 5.019 | 5.757 | 0.178 | 0.188 |
| *F.succinogenes* | 5.826 | 5.568 | 5.771 | 5.694 | 5.722 | 0.092 | 0.945 |
| *S.ruminantium* | 6.157 | 5.749 | 6.457 | 6.09 | 6.249 | 0.136 | 0.62 |
| *V. parvula* | 4.132 | 3.776 | 3.733 | 3.648 | 3.788 | 0.1 | 0.646 |
| *S.dextrinisolvens* | 4.99 | 6.202 | 5.39 | 5.665 | 6.037 | 0.175 | 0.173 |
| Fungi | 3.709 | 3.756 | 3.485 | 4.296 | 3.602 | 0.116 | 0.211 |
| Total bacterial | 7.015 | 6.637 | 7.17 | 5.876 | 6.75 | 0.183 | 0.196 |

Additional file 2: Table S5

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Function Prediction | C | LT | LW | MW | HW | SEM | *p-*value |
| Organism Systems | 0.79 | 0.79 | 0.8 | 0.75 | 0.75 | 0.01 | 0.28 |
| Genetic Information Processing | 21.47 | 21.41 | 21.42 | 21.15 | 21.13 | 0.06 | 0.27 |
| Cellular Processes | 2.47 | 2.59 | 2.63 | 2.81 | 2.92 | 0.08 | 0.38 |
| Environmental Information Processing | 10.13 | 10.34 | 10.43 | 11.02 | 10.95 | 0.23 | 0.74 |
| Metabolism | 50.24 | 49.88 | 49.75 | 49.4 | 49.53 | 0.22 | 0.81 |
| Human Diseases | 0.75 | 0.74 | 0.75 | 0.75 | 0.74 | 0.01 | 0.91 |
| Unclassified | 13.97 | 14.06 | 14.02 | 13.93 | 13.78 | 0.04 | 0.25 |
| Others | 0.19 | 0.19 | 0.19 | 0.2 | 0.2 | 0 | 0.26 |

Additional file 2: Table S6

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | C | LT | LW | MW | HW | SEM | *p*-value |
| Circulatory System | 0 | 0 | 0 | 0 | 0 | 0 | 0.42 |
| Digestive System | 0.08 | 0.09 | 0.08 | 0.06 | 0.06 | 0 | 0.29 |
| Endocrine System | 0.35 | 0.34 | 0.35 | 0.32 | 0.32 | 0.01 | 0.31 |
| Nervous System | 0.11 | 0.11 | 0.11 | 0.1 | 0.1 | 0 | 0 |
| Sensory System | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Excretory System | 0.03 | 0.02 | 0.02 | 0.02 | 0.03 | 0 | 0.06 |
| Immune System | 0.09 | 0.09 | 0.1 | 0.09 | 0.09 | 0 | 0.76 |
| Replication and Repair | 9.98 | 9.94 | 9.96 | 9.78 | 9.75 | 0.04 | 0.31 |
| Translation | 6.47 | 6.42 | 6.39 | 6.36 | 6.37 | 0.02 | 0.54 |
| Transcription | 2.41 | 2.48 | 2.48 | 2.43 | 2.43 | 0.02 | 0.89 |
| Folding, Sorting and Degradation | 2.65 | 2.62 | 2.63 | 2.63 | 2.62 | 0.01 | 0.97 |
| Cell Communication | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cell Motility | 1.52 | 1.68 | 1.73 | 1.88 | 1.98 | 0.08 | 0.47 |
| Cell Growth and Death | 0.6 | 0.61 | 0.6 | 0.61 | 0.6 | 0 | 0.96 |
| Transport and Catabolism | 0.35 | 0.3 | 0.31 | 0.33 | 0.35 | 0.01 | 0.25 |
| Environmental Adaptation | 0.14 | 0.14 | 0.15 | 0.15 | 0.15 | 0 | 0.56 |
| Membrane Transport | 8.73 | 8.95 | 9.01 | 9.51 | 9.44 | 0.21 | 0.77 |
| Signaling Molecules and Interaction | 0.18 | 0.17 | 0.18 | 0.16 | 0.17 | 0 | 0.27 |
| Signal Transduction | 1.24 | 1.24 | 1.26 | 1.38 | 1.36 | 0.03 | 0.4 |
| Metabolism of Terpenoids and Polyketides | 1.81 | 1.8 | 1.81 | 1.78 | 1.78 | 0.01 | 0.84 |
| Metabolism of Other Amino Acids | 1.61 | 1.62 | 1.6 | 1.61 | 1.59 | 0.01 | 0.97 |
| Carbohydrate Metabolism | 10.25 | 10.11 | 10.14 | 9.97 | 10.12 | 0.05 | 0.53 |
| Biosynthesis of Other Secondary Metabolites | 1.1 | 1.09 | 1.08 | 1.06 | 1.07 | 0.01 | 0.89 |
| Energy Metabolism | 6.04 | 6.02 | 5.97 | 6.01 | 6.1 | 0.03 | 0.61 |
| Glycan Biosynthesis and Metabolism | 3.05 | 2.95 | 2.97 | 2.94 | 2.89 | 0.05 | 0.94 |
| Metabolism of Co-factors and Vitamins | 4.76 | 4.86 | 4.81 | 4.75 | 4.7 | 0.04 | 0.86 |
| Amino Acid Metabolism | 10.69 | 10.64 | 10.51 | 10.5 | 10.52 | 0.06 | 0.79 |
| Xenobiotics Bio-degradation and Metabolism | 1.55 | 1.54 | 1.56 | 1.51 | 1.51 | 0.01 | 0.39 |
| Lipid Metabolism | 2.69 | 2.6 | 2.65 | 2.72 | 2.71 | 0.02 | 0.3 |
| Nucleotide Metabolism | 4.52 | 4.51 | 4.51 | 4.4 | 4.4 | 0.03 | 0.37 |
| Enzyme Families | 2.26 | 2.25 | 2.23 | 2.25 | 2.24 | 0.01 | 0.83 |
| Cardiovascular Diseases | 0 | 0 | 0 | 0 | 0 | 0 | 0.31 |
| Immune System Diseases | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0 | 0.57 |
| Infectious Diseases | 0.38 | 0.39 | 0.39 | 0.38 | 0.37 | 0 | 0.52 |
| Metabolic Diseases | 0.12 | 0.11 | 0.11 | 0.11 | 0.12 | 0 | 0.14 |
| Neurodegenerative Diseases | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0.59 |
| Cancers | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0 | 0.49 |
| Poorly Characterized | 4.83 | 4.81 | 4.81 | 4.84 | 4.79 | 0.01 | 0.81 |
| Genetic Information Processing | 2.67 | 2.67 | 2.69 | 2.67 | 2.67 | 0.01 | 0.9 |
| Cellular Processes and Signaling | 3.94 | 4 | 3.95 | 3.97 | 3.85 | 0.02 | 0.22 |
| Metabolism | 2.56 | 2.62 | 2.6 | 2.48 | 2.49 | 0.02 | 0.18 |

Additional file 2: Table S7

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | C | LT | LW | MW | HW | SEW | *P*-value |
| Alanine, aspartate and glutamate metabolism | 1.22 | 1.23 | 1.22 | 1.18 | 1.19 | 0.01 | 0.64 |
| Amino acid related enzymes | 1.64 | 1.64 | 1.62 | 1.63 | 1.62 | 0.01 | 0.93 |
| Amino sugar and nucleotide sugar metabolism | 1.38 | 1.39 | 1.39 | 1.39 | 1.4 | 0 | 0.91 |
| Aminoacyl-tRNA biosynthesis | 1.31 | 1.3 | 1.29 | 1.28 | 1.29 | 0 | 0.26 |
| ABC transporters | 2.29 | 2.38 | 2.37 | 2.58 | 2.54 | 0.07 | 0.68 |
| Carbon fixation pathways in prokaryotes | 1.16 | 1.13 | 1.13 | 1.11 | 1.13 | 0.01 | 0.57 |
| Cysteine and methionine metabolism | 1.05 | 1.06 | 1.03 | 1.04 | 1.03 | 0.01 | 0.73 |
| General function prediction only | 3.62 | 3.63 | 3.6 | 3.6 | 3.6 | 0.01 | 0.71 |
| DNA repair and recombination proteins | 3.21 | 3.2 | 3.21 | 3.13 | 3.12 | 0.02 | 0.29 |
| Function unknown | 1.21 | 1.18 | 1.21 | 1.24 | 1.19 | 0.01 | 0.28 |
| Glycolysis / Gluconeogenesis | 1.08 | 1.05 | 1.06 | 1.03 | 1.06 | 0.01 | 0.38 |
| Chaperones and folding catalysts | 1.12 | 1.11 | 1.12 | 1.09 | 1.09 | 0.01 | 0.84 |
| Glycine, serine and threonine metabolism | 0.97 | 0.98 | 0.96 | 0.94 | 0.94 | 0.01 | 0.81 |
| Energy metabolism | 1.06 | 1.06 | 1.06 | 1.02 | 1.03 | 0.01 | 0.61 |
| Chromosome | 1.69 | 1.7 | 1.7 | 1.69 | 1.66 | 0.01 | 0.39 |
| Fructose and mannose metabolism | 0.86 | 0.87 | 0.87 | 0.83 | 0.84 | 0.01 | 0.33 |
| Arginine and proline metabolism | 1.32 | 1.32 | 1.3 | 1.28 | 1.3 | 0.01 | 0.6 |
| DNA replication proteins | 1.38 | 1.37 | 1.38 | 1.34 | 1.35 | 0.01 | 0.4 |
| Homologous recombination | 1.1 | 1.08 | 1.09 | 1.08 | 1.07 | 0 | 0.49 |
| Nitrogen metabolism | 0.7 | 0.73 | 0.71 | 0.71 | 0.71 | 0 | 0.45 |
| One carbon pool by folate | 0.8 | 0.82 | 0.8 | 0.77 | 0.77 | 0.01 | 0.68 |
| Mismatch repair | 0.92 | 0.92 | 0.92 | 0.9 | 0.9 | 0 | 0.26 |
| Other ion-coupled transporters | 1.2 | 1.25 | 1.21 | 1.22 | 1.16 | 0.02 | 0.49 |
| Methane metabolism | 1.23 | 1.18 | 1.18 | 1.22 | 1.26 | 0.01 | 0.28 |
| Oxidative phosphorylation | 1.28 | 1.24 | 1.24 | 1.29 | 1.3 | 0.01 | 0.37 |
| Peptidoglycan biosynthesis | 0.93 | 0.94 | 0.94 | 0.9 | 0.89 | 0.01 | 0.22 |
| Pyruvate metabolism | 1 | 0.96 | 0.98 | 0.96 | 0.99 | 0.01 | 0.59 |
| Peptidases | 2.04 | 2.04 | 2.02 | 2.01 | 2 | 0.01 | 0.67 |
| Pyrimidine metabolism | 2.11 | 2.1 | 2.1 | 2.04 | 2.06 | 0.01 | 0.33 |
| Pentose phosphate pathway | 0.75 | 0.74 | 0.75 | 0.74 | 0.75 | 0.01 | 0.99 |
| Ribosome Biogenesis | 1.52 | 1.51 | 1.51 | 1.54 | 1.51 | 0.01 | 0.57 |
| Purine metabolism | 2.41 | 2.41 | 2.41 | 2.36 | 2.35 | 0.01 | 0.41 |
| Starch and sucrose metabolism | 1.01 | 1.04 | 1.03 | 0.98 | 1 | 0.01 | 0.65 |
| Ribosome | 2.83 | 2.81 | 2.79 | 2.73 | 2.75 | 0.02 | 0.39 |
| Secretion system | 1.06 | 1.07 | 1.11 | 1.14 | 1.11 | 0.01 | 0.5 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 1.04 | 1.04 | 1.01 | 1.01 | 1.01 | 0.01 | 0.7 |
| Transporters | 4.57 | 4.69 | 4.7 | 4.95 | 4.99 | 0.12 | 0.83 |
| Two-component system | 1.11 | 1.11 | 1.14 | 1.24 | 1.23 | 0.03 | 0.45 |
| Transcription factors | 1.21 | 1.26 | 1.25 | 1.23 | 1.24 | 0.03 | 0.99 |
| Transcription machinery | 1.02 | 1.04 | 1.04 | 1.01 | 1.01 | 0.01 | 0.44 |
| Translation proteins | 1.02 | 1.01 | 1.01 | 1 | 1 | 0 | 0.23 |

Additional file 2: Table S8

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Fermentation Parameter | cold and wind chill | | | | | SEM | *P* values |
| C | LT | LW | MW | HW |
| Acetate acid (mmol/L) | 52.4 | 55.52 | 48.44 | 35.33 | 25.5 | 3.42 | 0.009 |
|
| The ratio of acetic acid to propionic acid | 2.54 | 2.77 | 2.67 | 2.88 | 3.11 | 0.08 | 0.233 |
|
| Propionic acid (mmol/L) | 21.41 | 21.09 | 18.28 | 12.49 | 8.12 | 1.64 | 0.018 |
|
| Isobutyric (mmol/L) | 0.69 | 0.47 | 0.59 | 1.13 | 0.99 | 0.07 | 0.003 |
|
| Butyrate (mmol/L) | 8.8 | 8.21 | 6.51 | 5.26 | 3.47 | 0.66 | 0.048 |
|
| Isovaleric (mmol/L) | 0.76 | 0.48 | 0.74 | 1.49 | 1.27 | 0.1 | 0.001 |
|
| Pentanoic acid (mmol/L) | 0.85 | 0.87 | 0.72 | 0.65 | 0.52 | 0.59 | 0.316 |
|
| Total VFA (mmol/L) | 84.91 | 86.63 | 75.28 | 56.35 | 39.88 | 5.62 | 0.016 |
|

Additional file 3: Table S9

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ADG | DMI | AD | IFN-γ | IL-2 | IL-6 | IL-4 | SOD | GSH-PX | CAT | T-AOC | MDA |
| *Brachybacterium* | 0.309 | -0.368 | -0.621\*\* | -0.161 | -0.349 | 0.252 | -0.063 | 0.195 | 0.224 | 0.344 | 0.211 | -0.159 |
| *Prevotellaceae\_UCG-003* | 0.294 | -0.083 | 0.414 | 0.708\*\* | 0.618\*\* | 0.409 | -0.33 | -0.646\*\* | -0.532\* | -0.664\*\* | -0.451\* | 0.534\* |
| *Lachnospiraceae\_XPB1014* | -0.18 | -0.088 | -0.244 | -0.247 | -0.252 | -0.536\* | 0.553\* | 0.151 | 0.509\* | 0.315 | 0.580\*\* | -0.369 |
| *Pseudobutyrivibrio* | -0.374 | 0.374 | 0.135 | -0.192 | -0.201 | -0.423 | 0.402 | 0.188 | 0.37 | 0.221 | 0.317 | -0.229 |
| *Ruminiclostridium\_1* | -0.525\* | 0.424 | 0.162 | -0.435 | -0.266 | -0.597\*\* | 0.229 | 0.375 | 0.266 | 0.094 | 0.332 | -0.395 |
| *Ruminococcaceae\_UCG-005* | -0.480\* | 0.434 | 0.192 | -0.367 | -0.259 | -0.481\* | -0.059 | 0.27 | 0.182 | 0.072 | 0.096 | -0.256 |
| *Solobacterium* | 0.523\* | -0.125 | 0.258 | 0.675\*\* | 0.541\* | 0.540\* | -0.335 | -0.636\*\* | -0.419 | -0.316 | -0.24 | 0.510\* |
| *Devosia* | 0.293 | -0.612\*\* | -0.654\*\* | -0.15 | -0.31 | 0.07 | 0.135 | 0.092 | 0.259 | 0.517\* | 0.378 | -0.087 |
| *Rhizobium* | 0.373 | -0.537\* | -0.690\*\* | -0.094 | -0.3 | 0.265 | -0.027 | 0.065 | 0.117 | 0.446\* | 0.188 | -0.044 |
| *Sphingomonas* | 0.242 | -0.461\* | -0.710\*\* | -0.291 | -0.421 | -0.085 | 0.327 | 0.247 | 0.506\* | 0.608\*\* | 0.602\*\* | -0.241 |
| *unclassified\_f\_\_Enterobacteriaceae* | 0.225 | -0.462\* | -0.507\* | -0.207 | -0.277 | -0.053 | 0.111 | 0.139 | 0.346 | 0.448\* | 0.409 | -0.129 |
| *Sphaerochaeta* | -0.479\* | 0.462\* | 0.633\*\* | 0.142 | 0.137 | -0.311 | 0.084 | -0.094 | -0.222 | -0.473\* | -0.041 | 0.014 |
| *norank\_c\_\_WCHB1-41* | -0.519\* | 0.461\* | 0.157 | -0.494\* | -0.376 | -0.493\* | 0.254 | 0.517\* | 0.36 | -0.101 | 0.11 | -0.144 |
| acetic acid | 0.719\*\* | -0.606\*\* | -0.425 | 0.292 | 0.178 | 0.593\*\* | -0.252 | -0.272 | -0.102 | 0.122 | -0.112 | 0.374 |
| propionic acid | 0.750\*\* | -0.506\* | -0.34 | 0.303 | 0.233 | 0.620\*\* | -0.346 | -0.323 | -0.175 | 0.064 | -0.211 | 0.412 |
| isobutyric acid | -0.500\* | 0.538\* | 0.583\*\* | -0.082 | 0.152 | -0.491\* | 0.19 | 0.143 | 0.112 | -0.385 | -0.056 | -0.124 |
| butyrate | 0.638\*\* | -0.438 | -0.187 | 0.358 | 0.242 | 0.545\* | -0.231 | -0.299 | -0.198 | -0.031 | -0.051 | 0.347 |
| isovalerate | -0.553\* | 0.545\* | 0.550\* | -0.135 | 0.112 | -0.533\* | 0.152 | 0.181 | 0.161 | -0.332 | -0.113 | -0.106 |
| valeric acid | 0.521\* | -0.372 | -0.194 | 0.189 | 0.153 | 0.379 | -0.161 | -0.158 | -0.107 | -0.007 | -0.78 | 0.272 |
| total VFA | 0.499\* | -0.373 | -0.287 | 0.146 | -0.035 | 0.613\*\* | -0.296 | -0.215 | -0.521\* | 0.096 | -0.239 | 0.323 |
| \*,P<0.05.\*\*,P<0.01 |  |  |  |  |  |  |  |  |  |  |  |  |