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**Response of rumen microbiota, and metabolic profiles of rumen fluid, liver and serum of goats to high-grain diets**

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**Figure S1** A venn diagram illustrating shared and unique operational taxonomic units (OTUs) at 3% dissimilarity level of the rumen microbiota in goats fed the hay and high-grain (HG) diet.



**Figure S2** Unique and mutual metabolites identified in the rumen, liver and serum of goats fed hay or high-grain (HG) diet.

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**Figure S3** The correlations between the common metabolites of rumen fluid, liver and serum in goats of fed the hay and high-grain diets. The green line between metabolites and bacterial genera indicated negative correlation and the pink line indicated positive correlation. The correlation coefficients that with a statistical *P* value less than 0.05 and the absolute value greater than 0.7 were used to construct the network graph.



**Figure S4.** The principal component analysis (PCA) and the partial least squares discriminant analysis (PLS-DA) of rumen fluid (A and B), liver (C and D) and serum (E and F) in goats fed hay and high-grain (HG) diets. Each point represents one sample in each group (red = HG group; green = hay group).



**Figure S5** The alterations in fold changes (the ratio of mean value of peak area obtained from the high-grain group and the hay group) of three amino acids in common pathway in rumen fluid, liver and serum of goats.

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**Figure S6** Proposed map illustrates significantly different main metabolites in the rumen fluid, liver and serum of the high-grain-fed goats compared to the hay-fed goats. GABA, γ-aminobutyric acid.

**Table S1** The effects of high grain diet on ruminal fermentation parameters in goats

|  |  |  |  |
| --- | --- | --- | --- |
| Item  | Hay | High-grain | *P*-value |
| Ruminal parameters  |  |  |  |
| pH  | 6.12 ±0.09  |  5.33±0.09  |  <0.001  |
| Total VFA, (mM)  | 93.90±1.25 | 116.16±4.25 | 0.001 |
| Acetate, (mM) | 70.24±0.88 | 63.12±1.89 | 0.009 |
| Propionate, (mM)  | 14.57±0.29 | 34.12±2.24 |  <0.001  |
| Butyrate, (mM) | 7.15±0.39 | 15.81±0.94 |  <0.001  |
| Isobutyrate, (mM) | 1.09±0.10 | 1.43±0.15 | 0.089 |
| Valerate, (mM) | 0.45±0.04 | 0.74±0.05 | 0.002 |
| Isovalerate, (mM) | 0.41±0.12 | 0.94±0.14 | 0.019 |
| Ace:Pro | 4.82±0.05 | 1.88±0.10 |  <0.001  |
| Acetate, (mol%) | 74.81±0.48 | 54.40±0.48 | <0.001 |
| Propionate, (mol%) | 15.51±0.18 | 29.29±1.30 | <0.001 |
| Butyrate, (mol%) | 7.61±0.39 | 13.62±0.66 | <0.001 |
| Isobutyrate, (mol%) | 1.16±0.09 | 1.24±0.14 | 0.646 |
| Valerate, (mol%) | 0.47±0.04 | 0.64±0.05 | 0.022 |
| Isovalerate, (mol%) | 0.43±0.12 | 0.82±0.14 | 0.066 |
| Free lipopolysaccharide, (EU/mL)  | 22,547±1,065  | 64,628 ±1,685  |  <0.001 |

VFA = volatile fatty acids; Ace:Pro = the ration of acetate to propionate.

**Table S2** Ingredient and nutrient composition of the diets of the experimental goats.

|  |  |
| --- | --- |
|  | **Diet** |
| **Item** | **Hay**  | **High-grain**  |
| *Diet ingredient, % DM*  |  |  |
| Chinese wildrye  | 81 | 30 |
| Alfalfa  | 15 | 0 |
| Corn  | 0 | 45 |
| Wheat  | 0 | 20 |
| Soybean meal  | 0 | 1.1 |
| Limestone  | 0.5 | 0.95 |
| Salt  | 0.8 | 0.65 |
| Calcium monohydrogen phosphate  | 1.7 | 1.2 |
| Premix  | 1 | 1 |
| Sodium Bicarbonate  | 0 | 0.1 |
| *Nutrient levels*  |  |  |
| Metabolic energy , MJ/kg DM  | 8.31 | 11.31 |
| Crude protein, % DM  | 10.06 | 10.06 |
| Crude fat, % DM  | 3.55 | 3.59 |
| Neutral detergent fiber , % DM | 57.01 | 25.23 |
| Acid detergent fiber , % DM  | 35.72 | 13.55 |
| Crude ash, % DM  | 10.62 | 6.52 |
| Starch, % DM  | —\* | 58.23 |

\*Not determined, but considered equal to 0.

**Table S3** Identifiedmetabolomics of rumen fluid, liver and serum from goats fed the hay diet and the high-grain diet

|  |  |  |  |
| --- | --- | --- | --- |
| **unique in rumen** | **unique in liver** | **rumen & liver & serum** | **rumen & liver** |
| 1-Monopalmitin | 2-Aminoethylphosphonic acid | 2-Ketoglutaric acid | 3-Hydroxypyridine |
| 2,5-Diaminovalerolactam | 2-Keto-L-gluconic acid | 3-Hydroxybutyric acid | 4-Aminobutyric acid |
| 2-Glycerophosphate | 5'-Adenosine monophosphate | Aminomalonic acid | Asparagine |
| 2-Hydroxybutyric acid | Beta-Aminoisobutyric acid | Arachidonic acid | Aspartic acid |
| 2-Hydroxyisovaleric acid | Beta-Glycerophospate | Creatinine | Beta-Alanine |
| 2-Keto-gluconic acid | Chlorphentermine | Cysteine | Glycerol |
| 3-Glycerophosphate | D-Arabinose | D-Fructose | Glycolic acid |
| 5-Hydroxyhexanoic acid | D-Galactonic acid lactone | D-Glucose | Hexadecanoic acid |
| 9,12-Octadecadienoic acid | D-Galactose | Glucose-6-phosphate | Inosine |
| 9H-Purin-6-amine | D-Glucitol  | Glutamic acid | **rumen & serum** |
| Allonic acid lactone | Histidine | Glyceric acid | Glucuronic acid  |
| Alpha-Aminobutyric acid | Inositol-3-phosphate | Glycine  | Uracil |
| Azelaic acid | Lactose | Hypoxanthine | **liver & serum** |
| Benzoate | Mannonic acid-lactone | Inositol | 1,3-Propanediol |
| Cadaverine  | Mercaptoacetic acid | Isoleucine | 2-Ethylhydracrylic acid |
| Caproic acid | Methyl-alpha-D-glucopyranoside | Lactate | Alpha-Aminoisobutyric acid |
| Chenodeoxycholic acid | N-Heptadecane | Leucine | Alpha-Glycerophosphate |
| Dodecanedioic acid | O-Phosphorylethanolamine | Lysine | Alpha-Hydroxy-N-butyric acid |
| Gluconic acid-1,4-lactone  | Pipecolic acid | Malic acid | Cholesterol |
| Homoserine | Ribitol | Maltose | Fumaric acid |
| Hydrocinnamic acid | **unique in serum** | Mannose | Heptadecanoic acid |
| Lyxose | 2-Iodoethyl laurate | Monomethyl phosphate | Linoleic acid |
| Monostearin | 3,7,11,15-Tetramethyl-hexadecanoic acid | Oleic acid | Phosphoric acid |
| Myo-inositol-2-phosphate | 3-Phenylpropionic acid | Ornithine | Pyrophosphoric acid |
| N-Acetyl glucosamine | Carbamic acid 2-dimethylamino-ethyl ester | Palmitoleic acid | Tricarballylic acid |
| Nicotinic acid | Cis-9-hexadecenoic acid ester | Phenylalanine | Uric acid |
| Nonanoic acid | Citric acid | Proline | Cystine |
| Oxalate | Gulono-1,4-lactone | Pyroglutamic acid | N-Heptadecane |
| Phenylethanolamine | Hexanoic acid | Pyruvate |  |
| Phosphate | Hippuric acid | Stearic acid |  |
| Putrescine  | Hormone | Threonine |  |
| Thymine | Methionine | Tryptophan |  |
| Uridine | Palmitic acid | Tyrosine |  |
| Xanthine | Scyllo-inositol | Urea |  |
|  |  | Valine |  |
|  |  | Ethanolamine |  |
|  |  | Glutamine  |  |
|  |  | Serine  |  |
|   |   | Alanine |   |

**Table S4** Identification of significantly different metabolites in rumen, liver and serum between goats fed the hay diet or high-grain diet

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ID | RT  | M/Z  | VIP | *P* value | FC |
| *rumen* |  |  |  |  |  |
| Organic acid  |  |  |  |  |  |
| Acetate | -- | -- | 1.147 | 0.015 | 0.899 |
| Benzoate | 6.89 | 179 | 1.204 | 0.005 | 0.337 |
| Butyrate | -- | -- | 1.412 | 0.000 | 2.212 |
| Dodecanedioic acid | 11.96 | 359 | 1.360 | 0.003 | 0.064 |
| Hydrocinnamic acid | 8.21 | 207 | 1.177 | 0.006 | 0.298 |
| Propionate | -- | -- | 1.414 | 0.001 | 2.342 |
| Valerate | -- | -- | 1.281 | 0.001 | 1.676 |
| Isovalerate | -- | -- | 1.067 | 0.020 | 2.302 |
| 2-Ketoglutaric acid | 9.24 | 198 | 1.093 | 0.036 | 3.797 |
| Nicotinic acid | 7.28 | 180 | 1.361 | 0.000 | 3.412 |
| Amino acids and derivatives |  |  |  |  |  |
| Alanine | 5.54 | 190 | 1.307 | 0.001 | 3.962 |
| Alpha-Aminobutyric acid | 6.25 | 130 | 1.351 | 0.001 | 4.753 |
| Aminomalonic acid | 8.6 | 320 | 1.098 | 0.015 | 4.008 |
| Cysteine | 9.15 | 220 | 1.142 | 0.010 | 3.192 |
| Glutamic acid | 9.53 | 246 | 1.273 | 0.002 | 4.691 |
| Glutamine  | 10.42 | 156 | 1.145 | 0.009 | 9.289 |
| Glycine  | 5.7 | 204 | 1.229 | 0.008 | 3.289 |
| Homoserine | 8.44 | 292 | 1.155 | 0.008 | 4.916 |
| Isoleucine | 7.32 | 218 | 1.092 | 0.016 | 2.048 |
| Leucine | 7.13 | 158 | 1.085 | 0.017 | 2.643 |
| Lysine | 11.22 | 434 | 1.356 | 0.000 | 8.790 |
| Ornithine | 9.48 | 142 | 1.221 | 0.004 | 3.697 |
| Phenylalanine | 9.61 | 218 | 1.190 | 0.005 | 3.669 |
| Pyroglutamic acid | 8.96 | 230 | 1.266 | 0.002 | 3.106 |
| Serine  | 7.83 | 218 | 1.092 | 0.032 | 3.071 |
| Threonine | 8.025 | 218 | 1.278 | 0.001 | 3.544 |
| Tryptophan | 12.71 | 202 | 1.206 | 0.004 | 9.681 |
| Tyrosine | 11.33 | 218 | 1.222 | 0.004 | 4.034 |
| Valine | 6.66 | 218 | 1.106 | 0.014 | 2.198 |
| Amines |  |  |  |  |  |
| Ethanolamine | 7.09 | 174 | 1.038 | 0.043 | 2.793 |
| Putrescine  | 10.26 | 174 | 1.187 | 0.020 | 7.957 |
| Nucleosides and derivatives |  |  |  |  |  |
| Hypoxanthine | 10.63 | 265 | 1.149 | 0.009 | 2.505 |
| Sugars |  |  |  |  |  |
| D-Fructose | 11.04 | 307 | 1.288 | 0.001 | 5.627 |
| D-Glucose | 11.15 | 319 | 1.191 | 0.005 | 3.664 |
| Maltose | 14.85 | 361 | 1.376 | 0.000 | 42.273 |
| Mannose | 11.11 | 319 | 1.184 | 0.006 | 4.754 |
| Others |  |  |  |  |  |
| 2,5-Diaminovalerolactam | 8.51 | 243 | 1.232 | 0.003 | 2.391 |
| 2-Glycerophosphate | 10.38 | 299 | 1.157 | 0.008 | 7.377 |
| 3-Hydroxypyridine | 4.78 | 152 | 1.185 | 0.017 | 0.542 |
| 9H-Purin-6-amine | 10.95 | 264 | 1.302 | 0.001 | 6.946 |
| Glycerol | 7.13 | 205 | 1.419 | 0.000 | 3.859 |
| Inositol | 12.15 | 318 | 1.228 | 0.013 | 5.273 |
| LPS | -- | -- | 1.474 | <0.0001 | 2.866 |
| Myo-inositol-2-phosphate | 13.63 | 318 | 1.246 | 0.002 | 3.554 |
| N-Acetyl glucosamine | 12.19 | 319 | 1.232 | 0.014 | 7.390 |
| Phosphate | 7.15 | 314 | 1.393 | 0.001 | 4.277 |
| Glucose-6-phosphate | 13.15 | 387 | 1.226 | 0.011 | 3.997 |
| *liver* |  |  |  |  |  |
| Organic acids |  |  |  |  |  |
| Heptadecanoic acid | 12.22 | 327 | 1.771 | 0.022 | 0.719 |
| Amino acids and derivatives |  |  |  |  |  |
| Alpha-Aminoisobutyric acid | 6.25 | 130 | 1.636 | 0.041 | 0.565 |
| Cystine | 12.99 | 218 | 1.693 | 0.032 | 3.231 |
| GABA | 8.98 | 174 | 1.757 | 0.048 | 2.793 |
| Glycine | 5.70 | 204 | 1.727 | 0.028 | 1.369 |
| Lysine | 11.22 | 174 | 1.633 | 0.041 | 0.733 |
| Pyroglutamic acid | 8.95 | 156 | 1.783 | 0.021 | 1.306 |
| Valine | 6.65 | 144 | 1.622 | 0.043 | 0.726 |
| Others |  |  |  |  |  |
| Adenosine monophosphate | 16.9 | 315 | 1.618 | 0.044 | 0.540 |
| Glycerol | 7.14 | 205 | 1.715 | 0.029 | 1.234 |
| *serum* |  |  |  |  |  |
| Organic acids |  |  |  |  |  |
| 3-Phenylpropionic acid | 8.21 | 104 | 1.708 | 0.001 | 0.308 |
| Tricarballylic acid | 10.15 | 377 | 1.287 | 0.034 | 2.999 |
| Amino acids and derivatives |  |  |  |  |  |
| 2-Ethylhydracrylic acid | 6.74 | 177 | 1.266 | 0.038 | 1.822 |
| Alpha-Aminoisobutyric acid | 6.25 | 130 | 1.284 | 0.034 | 0.576 |
| Aminomalonic acid | 8.6 | 320 | 1.666 | 0.007 | 4.052 |
| Glycine | 5.70 | 204 | 1.744 | 0.001 | 2.367 |
| Hippuric acid | 10.61 | 105 | 1.615 | 0.011 | 0.255 |
| Isoleucine | 7.31 | 158 | 1.580 | 0.003 | 0.658 |
| Lysine | 11.22 | 156 | 1.433 | 0.013 | 0.520 |
| Proline | 7.34 | 142 | 1.283 | 0.035 | 1.406 |
| Threonine | 8.02 | 219 | 1.317 | 0.028 | 0.716 |
| Tyrosine | 11.32 | 218 | 1.244 | 0.043 | 1.659 |
| Valine | 6.65 | 144 | 1.488 | 0.008 | 0.603 |
| Sugars |  |  |  |  |  |
| D-Fructose | 11.03 | 217 | 1.374 | 0.020 | 1.610 |
| Lipids |  |  |  |  |  |
| Cholesterol  | 17.7 | 458 | 1.217 | 0.049 | 1.221 |
| Nucleosides |  |  |  |  |  |
| Hypoxanthine | 10.61 | 265 | 1.226 | 0.047 | 1.613 |
| Others |  |  |  |  |  |
| Phosphoric acid | 7.16 | 299 | 1.322 | 0.028 | 1.508 |

RT = retention time; M/Z = mass-to-charge ratio; VIP = the variable importance for the projection; FC = fold change, the ratio of mean value of peak area obtained from goats fed the high-grain diet and the mean value of peak area obtained from goats fed the hay diet; LPS = lipopolysaccharide; GABA =γ-aminobutyric acid.

Note: volatile fatty acid (--) and LPS (--) are detected by gas chromatography and assay kit respectively.