**Supplementary Material**

Table 1: Correlation matrix between rumen fermentation variables affected by methane inhibitors using a rumen simulation technique. Statistical levels were adjusted with the false discovery rate option.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Item1 | Dissolved H2 (μM) | Total gas (mL/d) | CH4 (mL/d) | H2 (mL/d) | CO2 (mL/d) | N2O (mL/d) | dOM (%) | EMPS2 | Total VFA (mM) | Acetate (mM) | Propionate (mM) |
| Total gas (mL/d) | -0.295 |  |  |  |  |  |  |  |  |  |  |
| CH4 (mL/d) | -0.195 | 0.506\* |  |  |  |  |  |  |  |  |  |
| H2 (mL/d) | -0.232 | 0.123 | -0.215 |  |  |  |  |  |  |  |  |
| CO2 (mL/d) | 0.023 | -0.539\* | -0.762\*\*\* | -0.469 |  |  |  |  |  |  |  |
| N2O (mL/d) | -0.103 | -0.470 | -0.575\* | -0.475 | 0.834\*\*\* |  |  |  |  |  |  |
| dOM (%) | 0.200 | 0.269 | 0.102 | 0.288 | -0.283 | -0.216 |  |  |  |  |  |
| EMPS2 | 0.067 | -0.511\* | -0.347 | -0.597\*\* | 0.709\*\* | 0.800\*\*\* | -0.376 |  |  |  |  |
| Total VFA (mM) | -0.077 | 0.498\* | 0.519\* | 0.290 | -0.663\*\* | -0.295 | 0.689\*\* | -0.454 |  |  |  |
| Acetate (mM) | -0.225 | -0.018 | 0.173 | -0.532\* | 0.196 | 0.508\* | 0.343 | 0.388 | 0.459 |  |  |
| Propionate (mM) | 0.227 | 0.309 | 0.216 | 0.440 | -0.488 | -0.210 | 0.800\*\*\* | -0.346 | 0.905\*\*\* | 0.405 |  |
| Isobutyrate (mM) | 0.257 | 0.517\* | 0.606\*\* | 0.427 | -0.832\*\*\* | -0.587\*\* | 0.640\*\* | -0.552\* | 0.877\*\*\* | 0.120 | 0.837\*\*\* |
| Butyrate (mM) | 0.049 | 0.472 | 0.355 | 0.597\*\* | -0.717\*\*\* | -0.469 | 0.748\*\*\* | -0.635\*\* | 0.921\*\*\* | 0.155 | 0.906\*\*\* |
| Valerate (mM) | 0.376 | 0.339 | 0.119 | 0.814\*\*\* | -0.647\*\* | -0.499\* | 0.601\*\* | -0.638\*\* | 0.689\*\* | -0.196 | 0.794\*\*\* |
| Isovalerate (mM) | 0.080 | 0.679\*\* | 0.769\*\*\* | 0.365 | -0.938\*\*\* | -0.786\*\*\* | 0.410 | -0.700\*\* | 0.721\*\*\* | -0.118 | 0.564\* |
| Caproate (mM) | -0.284 | 0.514\* | 0.426 | 0.562\* | -0.758\*\*\* | -0.635\*\* | 0.011 | -0.737\*\*\* | 0.395 | -0.494 | 0.175 |
| Heptanoate (mM) | -0.284 | 0.514\* | 0.426 | 0.563\* | -0.758\*\*\* | -0.636\*\* | 0.012 | -0.737\*\*\* | 0.395 | -0.494 | 0.175 |
| Ethanol (mM) | 0.301 | -0.565\* | -0.705\*\* | -0.110 | 0.711\*\* | 0.547\* | 0.129 | 0.561\* | -0.360 | 0.285 | -0.032 |
| NH4+ (mM) | -0.035 | 0.428 | 0.122 | 0.834\*\*\* | -0.663\*\* | -0.655\*\* | 0.308 | -0.835\*\*\* | 0.441 | -0.513\* | 0.400 |
| Formate (mM) | 0.365 | 0.058 | 0.311 | 0.596\*\* | -0.676\*\* | -0.692\*\* | 0.138 | -0.573\* | 0.196 | -0.576\* | 0.206 |
| Propanol (mM) | 0.585\* | -0.021 | -0.353 | 0.760\*\*\* | -0.185 | -0.202 | 0.546\* | -0.256 | 0.303 | -0.184 | 0.615\*\* |

1 \* *P* ≤ 0.10, \*\* *P* ≤ 0.05, \*\*\* *P* ≤ 0.01

2 Efficiency of microbial protein synthesis expressed as grams microbial N produced per kilogram OM fermented

Table 1 (end): Correlation matrix between rumen fermentation variables affected by methane inhibitors using a rumen simulation technique. Statistical levels were adjusted with the false discovery rate option.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Item1 | Isobutyrate (mM) | Butyrate (mM) | Valerate (mM) | Isovalerate (mM) | Caproate (mM) | Heptanoate (mM) | Ethanol (mM) | NH4+ (mM) | Formate (mM) |
| Butyrate (mM) | 0.900\*\*\* |  |  |  |  |  |  |  |  |
| Valerate (mM) | 0.813\*\*\* | 0.870\*\*\* |  |  |  |  |  |  |  |
| Isovalerate (mM) | 0.901\*\*\* | 0.748\*\*\* | 0.670\*\* |  |  |  |  |  |  |
| Caproate (mM) | 0.473 | 0.515\* | 0.546\* | 0.683\*\* |  |  |  |  |  |
| Heptanoate (mM) | 0.473 | 0.516\* | 0.547\* | 0.683\*\* | 1.000\*\*\* |  |  |  |  |
| Ethanol (mM) | -0.439 | -0.316 | -0.269 | -0.719\*\*\* | -0.856\*\*\* | -0.856\*\*\* |  |  |  |
| NH4+ (mM) | 0.546\* | 0.696\*\* | 0.771\*\*\* | 0.613\*\* | 0.838\*\*\* | 0.838\*\*\* | -0.516\* |  |  |
| Formate (mM) | 0.494 | 0.383 | 0.630\*\* | 0.559\* | 0.593\*\* | 0.593\*\* | -0.428 | 0.552\* |  |
| Propanol (mM) | 0.445 | 0.550\* | 0.746\*\*\* | 0.182 | 0.037 | 0.038 | 0.353 | 0.456 | 0.375 |

1 \* *P* ≤ 0.10, \*\* *P* ≤ 0.05, \*\*\* *P* ≤ 0.01

Figure 1: In vitro (rumen simulation technique) effect of a control treatment (CON) and nitrate (NIT), 3-nitrooxypropanol (NOP) and anthraquinone (AQ) on the relationship between gaseous H2 and CH4 production.