**Effect of adsorbants on *in vitro* biohydrogenation of 22:6n-3 by mixed cultures of rumen microorganisms**

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**Supplementary Table S1**. The effect of mucin, gum arabic, bentonite and silicic acid on the production of CH4 and apparent production of volatile fatty acids (µmol/flask) during 24 h incubations with mixed rumen fluid and 22:6n-3.1

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Amount  (mg/mL) | CH4 | Acetate | Propionate | Iso-butyrate | Butyrate | Iso-valerate | Valerate | Total VFA |
| Control |  | 259 | 421 | 93 | 28.1 | 65.5 | 63.3 | 56.0 | 733 |
| Mucin | 1 | 306 | 527 | 116 | 29.3 | 73.7 | 64.3 | 64.0 | 881 |
|  | 5 | 467 | 996 | 246 | 38.8 | 128.9 | 80.5 | 104.1 | 1605 |
| Gum arabic | 1 | 302 | 533 | 163 | 25.9 | 69.7 | 57.5 | 56.0 | 912 |
|  | 5 | 396 | 915 | 411 | 21.4 | 91.6 | 43.9 | 59.4 | 1545 |
| Bentonite | 1 | 261 | 407 | 87 | 26.7 | 60.7 | 59.9 | 54.1 | 702 |
|  | 5 | 241 | 413 | 89 | 26.6 | 60.2 | 58.3 | 53.7 | 707 |
| Silicic acid | 1 | 252 | 388 | 83 | 26.7 | 60.7 | 58.7 | 53.7 | 741 |
|  | 5 | 254 | 463 | 102 | 29.0 | 68.5 | 63.1 | 58.0 | 790 |
| SEM |  | 44.2 | 69.8 | 15.4 | 2.75 | 11.92 | 6.80 | 4.83 | 104.3 |
| P-value 2 | mucin | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|  | gum arabic | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.217 | <0.001 |
|  | bentonite | 0.415 | 0.938 | 0.858 | 0.335 | 0.534 | 0.170 | 0.545 | 0.815 |
|  | silicic acid | 0.901 | 0.236 | 0.284 | 0.190 | 0.446 | 0.630 | 0.317 | 0.428 |

1 The initial 22:6n-3 concentration was 0.02 mg/mL. Values represent least square means (n = 3)

2 Significance of linear components of the response to adsorbant concentration

**Supplementary Table S2.** The effect of mucin, gum arabic, bentonite and silicic acid on production of CH4 and apparent production of volatile fatty acids (µmol/flask) during 24h incubations with mixed rumen fluid and initial 22:6n-3.1

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Amount  (mg/mL) | CH4 | Acetate | Propionate | Iso-butyrate | Butyrate | Iso-valerate | Valerate | Total VFA |
| Control |  | 183 | 357 | 91.5 | 26.6 | 58.7 | 52.3 | 61.0 | 663 |
| Mucin | 1 | 250 | 519 | 140 | 30.4 | 79.4 | 66.0 | 75.7 | 922 |
|  | 5 | 389 | 891 | 265 | 38.7 | 125.5 | 80.9 | 112.2 | 1528 |
| Gum arabic | 1 | 186 | 469 | 184 | 25.2 | 68.3 | 55.5 | 61.8 | 868 |
|  | 5 | 317 | 868 | 444 | 23.7 | 101.2 | 51.2 | 65.8 | 1558 |
| Bentonite | 1 | 144 | 348 | 83.9 | 26.3 | 57.7 | 59.0 | 59.4 | 643 |
|  | 5 | 164 | 374 | 87.8 | 26.9 | 60.9 | 56.9 | 59.1 | 677 |
| Silicic acid | 1 | 186 | 343 | 88.9 | 26.4 | 58.2 | 58.6 | 60.3 | 644 |
|  | 5 | 187 | 364 | 92.2 | 27.1 | 61.9 | 60.0 | 60.6 | 675 |
| SEM |  | 45.5 | 70.4 | 15.7 | 2.71 | 10.83 | 6.21 | 5.26 | 107.0 |
| P-value 2 | mucin | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|  | gum arabic | <0.001 | <0.001 | <0.001 | 0.015 | <0.001 | 0.020 | 0.120 | <0.001 |
|  | bentonite | 0.401 | 0.662 | 0.935 | 0.646 | 0.687 | 0.774 | 0.631 | 0.751 |
|  | silicic acid | 0.888 | 0.794 | 0.911 | 0.520 | 0.606 | 0.732 | 0.946 | 0.787 |

1 The initial 22:6n-3 concentration was 0.06 mg/mL. Values represent least square means (n = 3)

2 Significance of linear components of the response to adsorbant concentration

**Supplementary Table S3.** The effect of mucin, gum arabic, bentonite and silicic acid on production of CH4 and apparent production of volatile fatty acids (µmol/flask) during 24 h incubations with mixed rumen fluid.1

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Amount  (mg/mL) | CH4 | Acetate | Propionate | Iso-butyrate | Butyrate | Iso-valerate | Valerate | Total VFA |
| Control |  | 148 | 289 | 78.4 | 24.0 | 45.4 | 54.6 | 61.0 | 564 |
| Mucin | 1 | 191 | 446 | 125.4 | 28.7 | 62.1 | 61.5 | 80.4 | 821 |
|  | 5 | 296 | 843 | 256.1 | 37.4 | 103.4 | 78.0 | 123.2 | 1465 |
| Gum arabic | 1 | 185 | 393 | 162.4 | 24.3 | 59.9 | 52.6 | 62.8 | 763 |
|  | 5 | 317 | 790 | 443.8 | 23.0 | 94.5 | 48.2 | 65.5 | 1468 |
| Bentonite | 1 | 143 | 333 | 88.4 | 24.0 | 51.8 | 55.9 | 60.8 | 623 |
|  | 5 | 163 | 354 | 88.8 | 26.5 | 55.7 | 59.1 | 61.4 | 655 |
| Silicic acid | 1 | 142 | 303 | 84.0 | 25.5 | 48.8 | 56.3 | 61.9 | 590 |
|  | 5 | 140 | 317 | 89.6 | 25.7 | 53.3 | 57.2 | 60.8 | 613 |
| SEM |  | 43.9 | 69.2 | 16.6 | 2.41 | 8.20 | 5.43 | 7.70 | 105.2 |
| P-value 2 | mucin | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|  | gum arabic | <0.001 | <0.001 | <0.001 | 0.503 | <0.001 | 0.020 | 0.547 | <0.001 |
|  | bentonite | 0.529 | 0.355 | 0.706 | 0.132 | 0.242 | 0.089 | 0.947 | 0.410 |
|  | silicic acid | 0.827 | 0.663 | 0.619 | 0.461 | 0.327 | 0.369 | 0.936 | 0.638 |

1 The initial 22:6n-3 concentration was 0.10 mg/mL. Values represent least square means (n = 3)

2 Significance of linear components of the response to adsorbant concentration

**Supplementary Table S4**. The effect of substrate amount and type on apparent production of volatile fatty acids (µmol/flask) during 24h incubations with mixed rumen fluid.1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment1 | Acetate | Propionate | Iso-butyrate | Butyrate | Iso-valerate | Valerate | Total VFA |
| Control - | 265a | 44a | 0.1a | 22a | 0.8a | 9a | 341a |
| Control + | 532b | 219b | 52.7c | 136b | 79.6d | 131b | 1164b |
| Mucin | 1381d | 525c | 81.3d | 280d | 115.3e | 223c | 2623d |
| Gum arabic | 1326d | 923d | 39.7b | 193bc | 51.2bc | 138b | 2677d |
| Starch | 1216d | 871d | 53.2c | 254d | 62.6cd | 131b | 2600d |
| Cellulose | 932c | 913d | 53.0c | 192bc | 60.3cd | 127b | 2283c |
| Xylan | 1388d | 975d | 39.7b | 220cd | 38.4b | 131b | 2797d |
| SEM | 47.0 | 46.6 | 2.75 | 22.1 | 5.11 | 2.6 | 64.5 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

1 The initial 22:6n-3 concentration was 0.06 mg/mL. Values represent least square means (n = 3)

2 control-: no carbohydrates present in the media, control+: control- with carbohydrate mixture (0.64 mg/mL), mucin, gum arabic, starch, cellulose, xylan: control+ with 5 mg/mL of the respective components

a,b,c,d,e indicates mean are different (*P* < 0.05)