***animal* journal**

**Dose-response effects of woody and herbaceous forage plants on *in vitro* ruminal methane and ammonia formation, and their short-term palatability in lactating cows**

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Supplementary Material

**Supplementary Table S1** *Feeding plan to determine the short-term palatability of the test plant pellets in the dairy cow experiment.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Duration | Animals | | | | | |
| (days) | Cow 1 | Cow 2 | Cow 3 | Cow 4 | Cow 5 | Cow 6 |
| 5 | Adaptation (lucerne pellets1) | | | | | |
| 3 | Control feeding (lucerne pellets1) | | | | | |
| 3 | Rosebay willow D | Wood avens C | Black-currant C | Vine D | Hazel C | Birch D |
| 3 | Birch C | Rosebay willow D | Wood avens D | Black-currant D | Vine D | Hazel C |
| 3 | Hazel D | Birch D | Rosebay willow C | Wood avens C | Black-currant D | Vine C |
| 3 | Vine C | Hazel C | Birch D | Rosebay willow C | Wood avens D | Black-currant C |
| 3 | Black-currant D | Vine D | Hazel D | Birch C | Rosebay willow C | Wood avens D |
| 3 | Wood avens C | Black-currant C | Vine C | Hazel D | Birch C | Rosebay willow D |

C, D = lot C and lot D, respectively

1Consisted (g/kg DM) of lucerne 980 (*Medicago sativa*; harvested in France 2016, purchased from Landi Sense Düdingen, Switzerland) and molasses 20.

**Supplementary Table S2** *Proportions of butyrate (C4) and valerate (C5) of total short-chain fatty acids (SCFA) in incubation fluid as affected by experimental plants in the Hohenheim gas test experiment.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | Dose (g/kg DM) | Basal diet | Birch | Sweet chest-nut | Hazel | Rose-bay willow | Wood avens | Black-currant | Vine | SEM | *P*-value | *P*-value | | |
|  | Plant | Dose | Plant × dose |
| C4 | 0 | 11.0 |  |  |  |  |  |  |  | 0.28 |  | <0.001 | <0.001 | <0.001 |
| (mmol/ | 100 |  | 11.1 | 11.2 | 11.1 | 11.3 | 11.2 | 11.2 | 11.1 |  | 0.521 |  |  |  |
| mol | 200 |  | 11.1 | 11.6 | 11.1 | 11.1 | 11.0 | 11.0 | 11.0 |  | 0.151 |  |  |  |
| SCFA) | 300 |  | 10.8b | 12.4\*a | 11.1b | 11.2b | 10.8b | 10.7b | 10.8b |  | <0.001 |  |  |  |
|  | 400 |  | 10.7b | 12.7\*a | 10.1\*b | 11.0b | 10.8b | 10.7b | 10.7b |  | <0.001 |  |  |  |
|  | 500 |  | 10.4b | 13.4\*a | 10.6b | 10.8b | 10.6b | 10.7b | 10.5b |  | <0.001 |  |  |  |
|  | 1000 |  | 8.7\*bc | 11.3a | 8.3\*c | 9.0\*b | 9.0\*b | 9.0\*b | 8.8\*bc |  | <0.001 |  |  |  |
|  | Contrast1 |  | L Q | L | L |  | L | L | L |  |  |  |  |  |
| iso C4 | 0 | 0.686 |  |  |  |  |  |  |  | 0.058 |  | <0.001 | <0.001 | <0.001 |
| (mmol/ | 100 |  | 1.04\*a | 1.00\*abc | 0.98\*bc | 0.94\*c | 0.95\*c | 0.96\*bc | 1.01\*ab |  | <0.001 |  |  |  |
| mol | 200 |  | 0.92\*a | 0.86ab | 0.82bc | 0.78c | 0.69d | 0.80bc | 0.81bc |  | <0.001 |  |  |  |
| SCFA) | 300 |  | 0.67a | 0.58ab | 0.54abc | 0.52bc | 0.44\*c | 0.50bc | 0.54bc |  | <0.001 |  |  |  |
|  | 400 |  | 0.40\*b | 0.48\*b | 0.49b | 0.84a | 0.90\*a | 0.87a | 0.82a |  | <0.001 |  |  |  |
|  | 500 |  | 0.74b | 0.54c | 0.63bc | 0.66bc | 0.93\*a | 0.70b | 0.68b |  | <0.001 |  |  |  |
|  | 1000 |  | 0.34\*b | 0.25\*b | 0.24\*b | 0.34\*b | 1.75\*a | 0.28\*b | 0.32\*b |  | <0.001 |  |  |  |
|  | Contrast |  | L Q | L Q | L | L | L Q |  | L |  |  |  |  |  |
| C5 | 0 | 1.06 |  |  |  |  |  |  |  | 0.039 |  | <0.001 | <0.001 | <0.001 |
| (mmol/ | 100 |  | 1.10 | 1.10 | 1.09 | 1.12 | 1.14 | 1.07 | 1.11 |  | 0.592 |  |  |  |
| mol | 200 |  | 1.07a | 1.07a | 1.00ab | 1.03ab | 0.99b | 1.01ab | 1.03ab |  | 0.019 |  |  |  |
| SCFA) | 300 |  | 0.95ab | 0.98a | 0.85\*b | 0.89\*ab | 0.93ab | 0.89\*ab | 0.91ab |  | 0.044 |  |  |  |
|  | 400 |  | 0.87\*ab | 0.94a | 0.74\*b | 0.97a | 0.98a | 0.92a | 0.97a |  | <0.001 |  |  |  |
|  | 500 |  | 0.88\*ab | 0.94a | 0.84\*ab | 0.88\*ab | 0.89\*ab | 0.79\*b | 0.89\*ab |  | 0.013 |  |  |  |
|  | 1000 |  | 0.53\*bc | 0.92a | 0.47\*c | 0.60\*bc | 0.69\*b | 0.53\*bc | 0.65\*bc |  | <0.001 |  |  |  |
|  | Contrast |  | L | L | L | L | L | L | L |  |  |  |  |  |
| iso C5 | 0 | 1.59 |  |  |  |  |  |  |  | 0.099 |  | <0.001 | <0.001 | <0.001 |
| (mmol/ | 100 |  | 1.66ab | 1.66ab | 1.67a | 1.59b | 1.64ab | 1.68a | 1.68a |  | 0.012 |  |  |  |
| mol | 200 |  | 1.67a | 1.49bc | 1.54b | 1.46bc | 1.43c | 1.54b | 1.49bc |  | <0.001 |  |  |  |
| SCFA) | 300 |  | 1.47b | 1.36\*ab | 1.38\*ab | 1.28\*bc | 1.21\*c | 1.32\*bc | 1.32\*bc |  | <0.001 |  |  |  |
|  | 400 |  | 1.24\*b | 1.28\*b | 1.50a | 1.50a | 1.52a | 1.69a | 1.53a |  | <0.001 |  |  |  |
|  | 500 |  | 1.71a | 1.49b | 1.33\*bc | 1.37\*bc | 1.30\*c | 1.50b | 1.38\*bc |  | <0.001 |  |  |  |
|  | 1000 |  | 1.30\*a | 1.13\*ab | 1.05\*ab | 0.92\*b | 0.911\*b | 1.02\*ab | 0.97\*b |  | 0.001 |  |  |  |
|  | Contrast |  | L Q | L Q | L | L Q | L Q | L Q | L |  |  |  |  |  |

a-dLeast-square means within a row with no common superscript differ (*P* < 0.05). DM = dry matter.

\*Values differ (*P* < 0.05) from those of basal diet alone.

1Significant (*P* < 0.05) linear (L) or quadratic (Q) contrasts of the response to incremental doses (from 0 to 500 g/kg) of each plant material.

**Supplementary Table S3** *Acetate/propionate ratio (C2/C3 ratio) as well as microbial counts in incubation fluid as affected by experimental plants in the Hohenheim gas test experiment.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | Dose (g/kg DM) | Basal diet | Birch | Sweet chest-nut | Hazel | Rose-bay willow | Wood avens | Black-currant | Vine | SEM | *P*-value | *P*-value | | |
|  | Plant | Dose | Plant × dose |
| C2/C3 | 0 | 3.53 |  |  |  |  |  |  |  | 0.089 |  | <0.001 | <0.001 | <0.001 |
| (x:1) | 100 |  | 3.43c | 3.42c | 3.52b | 3.49b | 3.49b | 3.53b | 3.59a |  | <0.001 |  |  |  |
|  | 200 |  | 3.55c | 3.45d | 3.69b | 3.72\*b | 3.67b | 3.72\*b | 3.83\*a |  | <0.001 |  |  |  |
|  | 300 |  | 3.67c | 3.40d | 3.84\*b | 3.89\*b | 3.82\*bc | 3.90\*b | 4.07\*a |  | <0.001 |  |  |  |
|  | 400 |  | 3.8\*b | 3.35\*e | 3.96\*ab | 3.90\*ab | 3.76\*b | 3.84\*b | 4.07\*a |  | <0.001 |  |  |  |
|  | 500 |  | 3.56d | 3.27\*e | 3.99\*bc | 4.06\*b | 3.89\*c | 3.99\*bc | 4.28\*a |  | <0.001 |  |  |  |
|  | 1000 |  | 3.52d | 3.40d | 4.59\*b | 5.10\*a | 4.37\*c | 4.71\*b | 5.21\*a |  | <0.001 |  |  |  |
|  | Contrast1 |  | L | L | L | L | L | L | L |  |  |  |  |  |
| Bacteria | 0 | 9.35 |  |  |  |  |  |  |  | 0.457 |  | 0.005 | 0.147 | 0.158 |
| (109/ml) | 100 |  | 8.68 | 8.84 | 8.99 | 8.80 | 8.65 | 8.93 | 8.72 |  | 0.993 |  |  |  |
|  | 200 |  | 8.53 | 9.08 | 8.91 | 9.68 | 9.24 | 9.96 | 8.83 |  | 0.169 |  |  |  |
|  | 300 |  | 9.45ab | 9.34ab | 8.26b | 8.61ab | 8.81ab | 9.34ab | 9.73a |  | 0.013 |  |  |  |
|  | 400 |  | 9.00 | 8.68 | 8.77 | 9.23 | 9.93 | 9.64 | 9.11 |  | 0.474 |  |  |  |
|  | 500 |  | 9.18 | 8.63 | 9.36 | 8.96 | 9.34 | 9.72 | 9.18 |  | 0.391 |  |  |  |
|  | 1000 |  | 8.59bc | 8.33c | 8.98abc | 10.3a | 9.54abc | 9.97ab | 9.82abc |  | 0.003 |  |  |  |
|  | Contrast |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Protozoa | 0 | 5.65 |  |  |  |  |  |  |  | 0.675 |  | 0.322 | <0.001 | 0.758 |
| (104/ml) | 100 |  | 5.04 | 4.41 | 3.74 | 4.74 | 4.30 | 4.44 | 4.89 |  | 0.698 |  |  |  |
|  | 200 |  | 5.56 | 4.93 | 5.15 | 5.96 | 4.19 | 5.30 | 4.19 |  | 0.567 |  |  |  |
|  | 300 |  | 4.44 | 3.89 | 4.04 | 4.30 | 5.11 | 5.78 | 4.59 |  | 0.224 |  |  |  |
|  | 400 |  | 5.07 | 4.93 | 5.19 | 4.85 | 5.30 | 5.93 | 5.04 |  | 0.783 |  |  |  |
|  | 500 |  | 5.00 | 4.19 | 4.59 | 5.22 | 4.48 | 4.04 | 3.67 |  | 0.081 |  |  |  |
|  | 1000 |  | 3.52 | 3.78 | 3.70 | 4.22 | 3.93 | 3.74 | 3.74 |  | 0.991 |  |  |  |
|  | Contrast |  |  |  |  |  |  |  | L |  |  |  |  |  |

a-eLeast-square means within a row with no common superscript differ (*P* < 0.05). DM = dry matter.

\*Values differ (*P* < 0.05) from those of basal diet alone.

1Significant (*P* < 0.05) linear (L) or quadratic (Q) contrasts of the response to incremental doses (from 0 to 500 g/kg) of each plant material.

**Supplementary Table S4** *Production of fermentation total gas, methane (CH4) and carbon dioxide (CO2) as affected by experimental plants in the Hohenheim gas test experiment.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | Dose (g/kg DM) | Basal diet | Birch | Sweet chest-nut | Hazel | Rose-bay willow | Wood avens | Black-currant | Vine | SEM | *P*-value | *P*-value | | |
|  | Plant | Dose | Plant × dose |
| Total gas | 0 | 45.8 |  |  |  |  |  |  |  | 1.09 |  | <0.001 | <0.001 | <0.001 |
| (ml/24h) | 100 |  | 44.8 | 44.0 | 43.7 | 42.7 | 42.0 | 42.1 | 43.3 |  | 0.681 |  |  |  |
|  | 200 |  | 40.6\* | 40.0\* | 41.2 | 40.3\* | 41.9 | 43.5 | 42.7 |  | 0.066 |  |  |  |
|  | 300 |  | 39.5\*ab | 35.0\*b | 36.4\*ab | 37.9\*ab | 40.4\*a | 39.0\*ab | 38.5\*ab |  | 0.020 |  |  |  |
|  | 400 |  | 38.4\*a | 27.4\*d | 34.6\*bc | 33.2\*c | 33.2\*a | 38.1\*a | 37.3\*ab |  | <0.001 |  |  |  |
|  | 500 |  | 34.3\*abc | 23.6\*d | 31.1\*c | 32.4\*bc | 32.4\*a | 35.7\*ab | 35.8\*ab |  | <0.001 |  |  |  |
|  | 1000 |  | 19.8\*d | 11.0\*f | 15.8\*e | 22.9\*cd | 33.5\*a | 26.4\*bc | 28.9\*b |  | <0.001 |  |  |  |
|  | Contrast1 |  | L | L Q | L | L | L | L | L |  |  |  |  |  |
| CH4 | 0 | 6.84 |  |  |  |  |  |  |  | 0.161 |  | <0.001 | <0.001 | <0.001 |
| (ml/24h) | 100 |  | 6.66 | 6.29 | 6.32 | 6.17\* | 6.20 | 6.25 | 6.37 |  | 0.258 |  |  |  |
|  | 200 |  | 5.93\*a | 5.29\*b | 5.80\*a | 5.74\*a | 5.92\*a | 6.14\*a | 6.06\*a |  | <0.001 |  |  |  |
|  | 300 |  | 5.59\*a | 3.73\*b | 5.00\*a | 5.37\*a | 4.95\*a | 5.59\*a | 5.58\*a |  | <0.001 |  |  |  |
|  | 400 |  | 5.15\*a | 2.35\*d | 4.62\*c | 4.64\*bc | 5.33\*a | 5.25\*a | 5.13\*ab |  | <0.001 |  |  |  |
|  | 500 |  | 4.56\*abc | 1.22\*d | 4.08\*c | 4.42\*bc | 5.05\*a | 4.83\*ab | 4.94\*a |  | <0.001 |  |  |  |
|  | 1000 |  | 1.90\*d | 0.23\*e | 1.66\*d | 2.74\*c | 4.04\*a | 3.26\*b | 3.34\*b |  | <0.001 |  |  |  |
|  | Contrast |  | L | L Q | L | L | L Q | L | L |  |  |  |  |  |
| CO2 | 0 | 38.4 |  |  |  |  |  |  |  | 1.06 |  | <0.001 | <0.001 | <0.001 |
| (ml/24h) | 100 |  | 37.8 | 36.9 | 36.8 | 35.7 | 35.5 | 35.5 | 36.6 |  | 0.716 |  |  |  |
|  | 200 |  | 34.0 | 34.2 | 34.8 | 34.2 | 35.1 | 36.8 | 35.7 |  | 0.316 |  |  |  |
|  | 300 |  | 33.4\* | 30.7\* | 30.3\* | 32.2\* | 31.1\* | 33.1\* | 32.6\* |  | 0.476 |  |  |  |
|  | 400 |  | 32.6\*a | 24.6\*c | 29.6\*ab | 28.0\*b | 32.7\*a | 32.4\*a | 31.2\*a |  | <0.001 |  |  |  |
|  | 500 |  | 29.5\*ab | 21.5\*c | 26.7\*b | 27.7\*b | 32.4\*a | 30.5\*ab | 30.5\*ab |  | <0.001 |  |  |  |
|  | 1000 |  | 17.5\*d | 10.6\*e | 13.7\*e | 19.7\*cd | 29.0\*a | 22.7\*bc | 25.3\*ab |  | <0.001 |  |  |  |
|  | Contrast |  | L | L Q | L | L | L | L | L |  |  |  |  |  |

a-fLeast-square means within a row with no common superscript differ (*P* < 0.05). DM = dry matter.

\*Values differ (*P* < 0.05) from those of basal diet alone.

1Significant (*P* < 0.05) linear (L) or quadratic (Q) contrasts of the response to incremental doses (from 0 to 500 g/kg) of each plant material.

**Supplementary Table S5** *Production of carbon dioxide (CO2) in relation to supply of dry matter (DM) and digestible organic matter (dOM) as affected by experimental plants in the Hohenheim gas test experiment.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Dose (g/kg DM) | Basal diet | Birch | Sweet chest-nut | Hazel | Rose-bay willow | Wood avens | Black-currant | Vine | SEM | *P*-value | *P*-value | | |
|  | Plant | Dose | Plant × dose |
| CO2/DM | 0 | 192 |  |  |  |  |  |  |  | 5.3 |  | <0.001 | <0.001 | <0.001 |
| (ml/g) | 100 |  | 189 | 185 | 184 | 179 | 177 | 177 | 183 |  | 0.716 |  |  |  |
|  | 200 |  | 170 | 171 | 174 | 171 | 175 | 184 | 178 |  | 0.316 |  |  |  |
|  | 300 |  | 167 | 154\* | 152\* | 161\* | 155\* | 166\* | 163\* |  | 0.476 |  |  |  |
|  | 400 |  | 163\*a | 123\*c | 148\*ab | 140\*b | 164\*a | 162\*a | 156\*a |  | <0.001 |  |  |  |
|  | 500 |  | 148\*ab | 108\*c | 134\*b | 138\*b | 162\*a | 153\*ab | 153\*ab |  | <0.001 |  |  |  |
|  | 1000 |  | 87\*d | 53\*e | 69\*e | 98\*cd | 145\*a | 114\*bc | 127\*ab |  | <0.001 |  |  |  |
|  | Contrast1 |  | L | L Q | L | L | L | L | L |  |  |  |  |  |
| CO2/dOM | 0 | 283 |  |  |  |  |  |  |  | 5.4 |  | <0.001 | <0.001 | <0.001 |
| (ml/g) | 100 |  | 281 | 278 | 279 | 273 | 274 | 273 | 278 |  | 0.684 |  |  |  |
|  | 200 |  | 269 | 275 | 273 | 271 | 272 | 278 | 274 |  | 0.803 |  |  |  |
|  | 300 |  | 269 | 267 | 255\* | 265 | 248\* | 265 | 264 |  | 0.539 |  |  |  |
|  | 400 |  | 271a | 248\*b | 258ab | 248\*b | 265a | 263a | 258ab |  | <0.001 |  |  |  |
|  | 500 |  | 256\*ab | 228\*c | 245\*bc | 247\*abc | 266a | 256\*ab | 256\*ab |  | <0.001 |  |  |  |
|  | 1000 |  | 199\*c | 156\*d | 172\*d | 211\*c | 255\*a | 217\*bc | 236\*ab |  | <0.001 |  |  |  |
|  | Contrast |  | L | L Q | L | L | L | L | L |  |  |  |  |  |

a-eLeast-square means within a row with no common superscript differ (*P* < 0.05).

\*Values differ (*P* < 0.05) from those of basal diet alone.

1Significant (*P* < 0.05) linear (L) or quadratic (Q) contrasts of the response to incremental doses (from 0 to 500 g/kg) of each plant material.