**Linking metabolites in eight bioactive forage species to their *in vitro* methane reduction potential across several cultivars and harvests**

Supriya Verma 1,\*, Siegfried Wolffram 2, Juha-Pekka Salminen 3, Mario Hasler4, Andreas Susenbeth 2, Ralf Blank 2 Friedhelm Taube 1,5, , Christof Kluß 1 and Carsten Stefan Malisch 1

1Institute of Plant Production and Plant Breeding, Grass and Forage Science/Organic Agriculture, Kiel University (CAU), 24118 Kiel, Germany

2Institute of Animal Nutrition and Physiology, Kiel University (CAU), 24118 Kiel, Germany;

3 Natural Chemistry Research Group, Department of Chemistry, University of Turku, Vatselankatu 2, FI-20014 Turku, Finland

4Department of Statistics, Kiel University (CAU), 24118 Kiel, Germany

5Grass Based Dairy Systems, Animal Production Systems Group, Wageningen University (WUR), 6705 Wageningen, The Netherlands

\* Corresponding author: Email address: sverma@gfo.uni-kiel.de, Tel: +49 431 880 2137

**Supplementary Table S1.** Gas production from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in gas production from the cultivar when compared with lucerne, Red\_PEG refers to reduction in gas production from the cultivar in the absence of polyethylene glycol (PEG, a tannin binding agent) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | **Harvest 2** |
| **-PEG** **(ml/200mg DM)** | **+PEG** **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG** **(ml/200mg DM)** | **+PEG** **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 47.93 | 48.12 | -6 | 0 | 44.30 | 44.07 | 2 | 1 |
| Chicory | Spadona | 54.42 | 53.40 | -20 | -2 | 51.35 | 51.65 | -15 | -1 |
| Sulla | Grimaldi | 31.27 | 42.11 | 31 | 26 | 45.20 | 32.48 | 28 | 28 |
| Sulla | Sudda | 26.26 | 46.75 | 42 | 44 | 40.78 | 25.62 | 43 | 37 |
| Birdsfoot trefoil | Bull | 45.25 | 47.67 | 0 | 5 | 51.54 | 50.10 | -11 | 3 |
| Birdsfoot trefoil | Lotar | 45.47 | 49.26 | -1 | 8 | 49.97 | 47.73 | -6 | 4 |
| Birdsfoot trefoil | Rocco | 43.40 | 45.50 | 4 | 5 | 47.93 | 45.05 | 0 | 6 |
| Big trefoil | Lot 29 | 27.54 | 43.14 | 39 | 36 | 43.60 | 35.60 | 21 | 18 |
| Big trefoil | Wild type | 31.27 | 41.63 | 31 | 25 | 46.08 | 42.38 | 6 | 8 |
| Lucerne | Galaxy | 45.17 | 48.10 | 0 | 6 | 47.35 | 44.78 | 1 | 5 |
| Sainfoin | CPI 63750 | 42.81 | 49.29 | 5 | 13 | 42.33 | 40.83 | 9 | 4 |
| Sainfoin | Esky | 38.74 | 48.69 | 14 | 20 | 42.77 | 39.56 | 12 | 7 |
| Sainfoin | Visnovsky | 36.89 | 46.82 | 18 | 21 | 44.75 | 37.63 | 16 | 16 |
| Plantain | PLA60 | 45.44 | 46.12 | -1 | 1 | 46.23 | 48.07 | -7 | -4 |
| Plantain | Svatojansky | 32.46 | 33.33 | 28 | 3 | 51.98 | 51.55 | -14 | 1 |
| Salad burnet | PI 308861 | 38.48 | 46.90 | 15 | 18 | 39.65 | 27.97 | 38 | 29 |
| Salad burnet | Sang 10 | 44.28 | 49.98 | 2 | 11 | 39.75 | 32.60 | 28 | 18 |

**Supplementary Table S2**. Methane production from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in methane production from the cultivar when compared with lucerne, Red\_PEG refers to reduction in methane production from the cultivar in the absence of polyethylene glycol (PEG, a tannin binding agent) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | **Harvest 2** |
| **-PEG** **(ml/200mg DM)** | **+PEG** **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG** **(ml/200mg DM)** | **PEG** **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 9.51 | 9.44 | 12 | -1 | 9.05 | 8.98 | 16 | -1 |
| Chicory | Spadona | 10.51 | 10.41 | 3 | -1 | 10.67 | 10.72 | 1 | 0 |
| Sulla | Grimaldi | 7.28 | 9.56 | 33 | 24 | 7.66 | 10.47 | 29 | 27 |
| Sulla | Sudda | 5.62 | 10.08 | 48 | 44 | 5.85 | 9.25 | 46 | 37 |
| Birdsfoot trefoil | Bull | 10.91 | 11.17 | -1 | 2 | 11.75 | 11.34 | -9 | -4 |
| Birdsfoot trefoil | Lotar | 11.02 | 11.53 | -2 | 4 | 12.20 | 12.40 | -13 | 2 |
| Birdsfoot trefoil | Rocco | 9.85 | 10.48 | 9 | 6 | 11.52 | 11.97 | -6 | 4 |
| Big trefoil | Lot 29 | 6.79 | 9.86 | 37 | 31 | 8.57 | 9.98 | 21 | 14 |
| Big trefoil | Wild type | 7.34 | 9.11 | 32 | 19 | 10.04 | 10.72 | 7 | 6 |
| Lucerne | Galaxy | 10.80 | 10.86 | 0 | 1 | 10.84 | 11.05 | 0 | 2 |
| Sainfoin | CPI 63750 | 9.44 | 10.47 | 13 | 10 | 9.30 | 9.88 | 14 | 6 |
| Sainfoin | Esky | 8.55 | 10.64 | 21 | 20 | 9.38 | 10.23 | 13 | 8 |
| Sainfoin | Visnovsky | 8.72 | 10.26 | 19 | 15 | 8.67 | 10.17 | 20 | 15 |
| Plantain | PLA60 | 8.67 | 8.93 | 20 | 3 | 9.93 | 9.33 | 8 | -6 |
| Plantain | Svatojansky | 5.59 | 5.73 | 48 | 3 | 10.47 | 10.38 | 3 | -1 |
| Salad burnet | PI 308861 | 7.93 | 9.92 | 27 | 20 | 7.05 | 8.98 | 35 | 22 |
| Salad burnet | Sang 10 | 9.12 | 10.67 | 16 | 15 | 7.73 | 8.87 | 29 | 13 |

**Supplementary Table S3**. Methane percentage in total gas (MP) from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in MP from the cultivar when compared with lucerne, Red\_PEG refers to reduction in MP from the cultivar in the absence of polyethylene glycol (-PEG) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | **Harvest 2** |
| **-PEG** **(%)** | **+PEG** **(%)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG** **(%)** | **+PEG** **(%)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 19.88 | 19.64 | 17 | -1 | 20.52 | 20.33 | 15 | -1 |
| Chicory | Spadona | 19.33 | 19.49 | 19 | 1 | 20.72 | 20.87 | 14 | 1 |
| Sulla | Grimaldi | 23.29 | 22.76 | 3 | -2 | 23.64 | 23.17 | 2 | -2 |
| Sulla | Sudda | 21.55 | 21.59 | 10 | 0 | 22.90 | 22.73 | 5 | -1 |
| Birdsfoot trefoil | Bull | 24.16 | 23.47 | -1 | -3 | 23.47 | 22.08 | 3 | -6 |
| Birdsfoot trefoil | Lotar | 24.46 | 23.45 | -2 | -4 | 25.57 | 24.78 | -6 | -3 |
| Birdsfoot trefoil | Rocco | 22.82 | 23.21 | 5 | 2 | 25.62 | 24.95 | -6 | -3 |
| Big trefoil | Lot 29 | 24.98 | 23.07 | -4 | -8 | 24.03 | 22.88 | 0 | -5 |
| Big trefoil | Wild type | 23.71 | 22.03 | 1 | -8 | 23.66 | 23.28 | 2 | -2 |
| Lucerne | Galaxy | 23.91 | 22.59 | 0 | -6 | 24.20 | 23.35 | 0 | -3 |
| Sainfoin | CPI 63750 | 22.13 | 21.32 | 7 | -4 | 22.82 | 23.40 | 6 | 2 |
| Sainfoin | Esky | 22.09 | 21.90 | 8 | -1 | 23.73 | 23.93 | 2 | 1 |
| Sainfoin | Visnovsky | 23.72 | 21.95 | 1 | -8 | 23.10 | 22.80 | 4 | -1 |
| Plantain | PLA60 | 19.07 | 19.36 | 20 | 1 | 20.67 | 20.35 | 14 | -2 |
| Plantain | Svatojansky | 17.19 | 17.20 | 28 | 0 | 20.35 | 19.98 | 16 | -2 |
| Salad burnet | PI 308861 | 20.70 | 21.20 | 13 | 2 | 25.29 | 22.67 | -5 | -12 |
| Salad burnet | Sang 10 | 20.61 | 21.45 | 14 | 4 | 23.75 | 22.30 | 2 | -7 |