Relationships between methane emission of Holstein Friesian dairy cows and fatty acids, volatile metabolites and non-volatile metabolites in milk.

S. van Gastelen, E.C. Antunes-Fernandes, K.A. Hettinga and J. Dijkstra

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table S1** *Linear regressions between methane production (g/d) and milk fatty acid concentrations* | | | | | | |
| Fatty acid (g/100 g fatty acids) | Intercept | SE | Slope | SE | Slope *P* | R2 |
| C4:0 | 427 | 79.5 | -6.2 | 23.57 | 0.80 | < 0.01 |
| C6:0 | 280 | 118.7 | 56.8 | 53.40 | 0.30 | 0.04 |
| C8:0 | 271 | 82.4 | 111.7 | 67.97 | 0.11 | 0.09 |
| C10:0 | 343 | 56.6 | 23.3 | 20.55 | 0.27 | 0.05 |
| C12:0 | 344 | 47.0 | 19.1 | 14.30 | 0.19 | 0.06 |
| C14:0 | 337 | 102.5 | 6.2 | 9.05 | 0.50 | 0.02 |
| *iso* C14:0 | 430 | 45.0 | -274.2 | 516.17 | 0.60 | 0.01 |
| C14:1 *cis*-9 | 341 | 30.1 | 53.8 | 24.08 | 0.03 | 0.16 |
| C15:0 | 351 | 35.4 | 52.7 | 33.26 | 0.13 | 0.09 |
| *iso* C15:0 | 341 | 46.9 | 258.1 | 184.36 | 0.17 | 0.07 |
| *anteiso* C15:0 | 390 | 60.0 | 40.5 | 144.18 | 0.78 | < 0.01 |
| C16:0 | 255 | 88.3 | 4.3 | 2.48 | 0.10 | 0.10 |
| *iso* C16:0 | 459 | 53.1 | -305.1 | 306.38 | 0.33 | 0.04 |
| C16:1 *trans*-9 | 442 | 43.2 | -172.1 | 205.65 | 0.41 | 0.03 |
| C16:1 *cis*-9 | 390 | 47.1 | 8.2 | 23.41 | 0.73 | < 0.01 |
| C17:0 | 380 | 52.4 | 42.7 | 84.50 | 0.62 | < 0.01 |
| *iso* C17:0 | 464 | 82.5 | -156.4 | 221.47 | 0.49 | 0.02 |
| *anteiso* C17:0 | 428 | 57.2 | -52.4 | 139.28 | 0.71 | < 0.01 |
| C17:1 *cis*-9 | 438 | 50.1 | -114.0 | 178.58 | 0.53 | 0.01 |
| C18:0 | 436 | 51.5 | -4.0 | 6.85 | 0.57 | 0.01 |
| C18:1 *cis*-9A | 467 | 45.0 | -3.4 | 2.50 | 0.18 | 0.06 |
| C18:1 *cis*-12 | 442 | 14.2 | -181.2 | 65.05 | < 0.01 | 0.22 |
| C18:1 *cis*-13 | 458 | 35.7 | -455.4 | 312.13 | 0.16 | 0.07 |
| C18:1 *trans*-6 | 442 | 19.9 | -160.6 | 84.48 | 0.07 | 0.12 |
| C18:1 *trans*-9 | 469 | 23.8 | -449.4 | 163.93 | 0.01 | 0.22 |
| C18:1 *trans*-10 | 434 | 11.4 | -122.9 | 43.35 | < 0.01 | 0.23 |
| C18:1 *trans*-11 | 466 | 15.1 | -70.5 | 16.65 | < 0.01 | 0.40 |
| C18:1 *trans*-15 + C18:1 *cis*-11 | 482 | 30.4 | -128.5 | 50.36 | 0.02 | 0.19 |
| C18:2 *cis*-9*, trans*-11 | 454 | 13.9 | -108.9 | 29.13 | < 0.01 | 0.34 |
| C18:2n-6 | 511 | 36.8 | -71.4 | 24.84 | < 0.01 | 0.23 |
| C18:3n-3 | 395 | 23.4 | 28.1 | 56.75 | 0.62 | < 0.01 |
| C18:3n-6 | 529 | 39.7 | -1611.9 | 517.80 | < 0.01 | 0.26 |
| C20:0 | 367 | 51.8 | 325.1 | 420.31 | 0.45 | 0.02 |
| C20:1 *cis*-11 | 445 | 34.1 | -854.3 | 733.46 | 0.25 | 0.05 |
| C20:2n-6 | 478 | 49.2 | -1535.2 | 1038.17 | 0.15 | 0.07 |
| C20:3n-6 | 482 | 33.3 | -843.9 | 362.83 | 0.03 | 0.17 |
| C20:4n-3 | 376 | 13.0 | 426.9 | 162.50 | 0.01 | 0.20 |
| C20:4n-6 | 496 | 34.2 | -683.4 | 265.95 | 0.01 | 0.21 |
| C20:5n-3 | 397 | 32.2 | 165.8 | 527.29 | 0.76 | < 0.01 |
| C22:0 | 385 | 20.0 | 343.0 | 302.07 | 0.27 | 0.05 |
| C22:5n-3 | 443 | 44.7 | -398.6 | 483.07 | 0.42 | 0.02 |
| C24:0 | 385 | 18.0 | 471.2 | 360.68 | 0.20 | 0.06 |
| A C18:1 cis-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible, as this FA is always present in small amounts. | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table S2** *Linear regressions between methane yield (g/kg DMIA) and milk fatty acid concentrations* | | | | | | |
| Fatty acid (g/100 g fatty acids) | Intercept | SE | Slope | SE | Slope *P* | R2 |
| C4:0 | 25.9 | 4.00 | -0.54 | 1.185 | 0.65 | < 0.01 |
| C6:0 | 23.1 | 6.10 | 0.45 | 2.746 | 0.87 | < 0.01 |
| C8:0 | 22.4 | 4.34 | 1.41 | 3.583 | 0.70 | < 0.01 |
| C10:0 | 23.4 | 2.92 | 0.24 | 1.059 | 0.82 | < 0.01 |
| C12:0 | 23.8 | 2.45 | 0.08 | 0.744 | 0.91 | < 0.01 |
| C14:0 | 25.9 | 5.20 | -0.15 | 0.459 | 0.74 | < 0.01 |
| *iso* C14:0 | 28.8 | 2.09 | -54.64 | 23.944 | 0.03 | 0.16 |
| C14:1 *cis*-9 | 24.9 | 1.65 | -0.65 | 1.315 | 0.63 | < 0.01 |
| C15:0 | 22.0 | 1.82 | 2.04 | 1.708 | 0.24 | 0.05 |
| *iso* C15:0 | 19.7 | 2.29 | 17.68 | 9.001 | 0.06 | 0.13 |
| *anteiso* C15:0 | 26.0 | 3.00 | -4.57 | 7.224 | 0.53 | 0.01 |
| C16:0 | 20.5 | 4.64 | 0.10 | 0.130 | 0.44 | 0.02 |
| *iso* C16:0 | 28.2 | 2.60 | -24.05 | 15.027 | 0.12 | 0.09 |
| C16:1 *trans*-9 | 26.5 | 2.15 | -11.50 | 10.262 | 0.27 | 0.04 |
| C16:1 *cis*-9 | 25.1 | 2.37 | -0.51 | 1.179 | 0.67 | < 0.01 |
| C17:0 | 17.8 | 2.35 | 10.31 | 3.791 | 0.01 | 0.22 |
| *iso* C17:0 | 22.4 | 4.18 | 4.60 | 11.230 | 0.69 | < 0.01 |
| *anteiso* C17:0 | 24.4 | 2.89 | -0.64 | 7.037 | 0.93 | < 0.01 |
| C17:1 *cis*-9 | 21.6 | 2.49 | 9.04 | 8.900 | 0.32 | 0.04 |
| C18:0 | 20.4 | 2.51 | 0.50 | 0.340 | 0.15 | 0.08 |
| C18:1 *cis*-9B | 23.7 | 2.34 | 0.02 | 0.130 | 0.85 | < 0.01 |
| C18:1 *cis*-12 | 26.8 | 0.58 | -13.62 | 2.640 | < 0.01 | 0.50 |
| C18:1 *cis*-13 | 27.0 | 1.78 | -26.00 | 15.555 | 0.11 | 0.09 |
| C18:1 *trans*-6 | 27.5 | 0.82 | -15.19 | 3.466 | < 0.01 | 0.42 |
| C18:1 *trans*-9 | 28.6 | 1.02 | -31.77 | 7.064 | < 0.01 | 0.43 |
| C18:1 *trans*-10 | 26.1 | 0.46 | -9.16 | 1.757 | < 0.01 | 0.50 |
| C18:1 *trans*-11 | 27.5 | 0.68 | -4.05 | 0.751 | < 0.01 | 0.52 |
| C18:1 *trans*-15 + C18:1 *cis*-11 | 26.9 | 1.62 | -4.68 | 2.681 | 0.09 | 0.10 |
| C18:2 *cis*-9*, trans*-11 | 27.2 | 0.58 | -6.99 | 1.208 | < 0.01 | 0.55 |
| C18:2n-6 | 29.8 | 1.80 | -3.91 | 1.217 | < 0.01 | 0.28 |
| C18:3n-3 | 22.0 | 1.10 | 5.38 | 2.680 | 0.05 | 0.13 |
| C18:3n-6 | 31.6 | 1.82 | -98.92 | 23.731 | < 0.01 | 0.39 |
| C20:0 | 16.2 | 2.15 | 64.77 | 17.417 | < 0.01 | 0.34 |
| C20:1 *cis*-11 | 23.0 | 1.75 | 24.64 | 37.587 | 0.52 | 0.02 |
| C20:2n-6 | 22.1 | 2.54 | 43.86 | 53.744 | 0.42 | 0.02 |
| C20:3n-6 | 26.7 | 1.77 | -28.64 | 19.262 | 0.15 | 0.08 |
| C20:4n-3 | 23.5 | 0.72 | 9.08 | 9.010 | 0.32 | 0.04 |
| C20:4n-6 | 29.3 | 1.66 | -39.30 | 14.429 | < 0.01 | 0.27 |
| C20:5n-3 | 22.7 | 1.60 | 24.30 | 26.211 | 0.36 | 0.03 |
| C22:0 | 21.7 | 0.90 | 39.23 | 13.633 | 0.01 | 0.23 |
| C22:5n-3 | 25.9 | 2.25 | -19.60 | 24.362 | 0.43 | 0.02 |
| C24:0 | 22.2 | 0.85 | 41.43 | 16.964 | 0.02 | 0.18 |
| A Dry matter intake (kg/d). | | | | | | |
| B C18:1 cis-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible, as this FA is always present in small amounts. | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table S3** *Linear regressions between methane intensity (g/kg FPCMA) and milk fatty acid concentrations* | | | | | | |
| Fatty acid (g/100 g fatty acids) | Intercept | SE | Slope | SE | Slope *P* | R2 |
| C4:0 | 22.3 | 3.95 | -1.75 | 1.171 | 0.15 | 0.08 |
| C6:0 | 18.7 | 6.24 | -1.03 | 2.809 | 0.72 | < 0.01 |
| C8:0 | 14.1 | 4.44 | 1.93 | 3.664 | 0.60 | 0.01 |
| C10:0 | 13.7 | 2.95 | 0.99 | 1.069 | 0.36 | 0.03 |
| C12:0 | 13.2 | 2.43 | 1.00 | 0.738 | 0.19 | 0.06 |
| C14:0 | 10.9 | 5.23 | 0.49 | 0.462 | 0.30 | 0.04 |
| *iso* C14:0 | 14.8 | 2.32 | 18.78 | 26.558 | 0.49 | 0.02 |
| C14:1 *cis*-9 | 13.1 | 1.56 | 2.74 | 1.247 | 0.04 | 0.15 |
| C15:0 | 12.5 | 1.75 | 3.74 | 1.646 | 0.03 | 0.16 |
| *iso* C15:0 | 9.2 | 2.08 | 28.75 | 8.165 | < 0.01 | 0.31 |
| *anteiso* C15:0 | 11.6 | 2.96 | 11.56 | 7.119 | 0.12 | 0.09 |
| C16:0 | 10.6 | 4.67 | 0.16 | 0.132 | 0.23 | 0.05 |
| *iso* C16:0 | 19.7 | 2.72 | -19.38 | 15.677 | 0.23 | 0.05 |
| C16:1 *trans*-9 | 20.7 | 2.10 | -20.77 | 9.990 | 0.05 | 0.14 |
| C16:1 *cis*-9 | 13.7 | 2.38 | 1.37 | 1.183 | 0.26 | 0.05 |
| C17:0 | 15.0 | 2.71 | 2.24 | 4.364 | 0.61 | < 0.01 |
| *iso* C17:0 | 16.1 | 4.30 | 0.82 | 11.545 | 0.94 | < 0.01 |
| *anteiso* C17:0 | 19.8 | 2.89 | -8.37 | 7.032 | 0.24 | 0.05 |
| C17:1 *cis*-9 | 18.5 | 2.57 | -7.67 | 9.176 | 0.41 | 0.03 |
| C18:0 | 19.4 | 2.62 | -0.40 | 0.348 | 0.26 | 0.05 |
| C18:1 *cis*-9B | 19.8 | 2.31 | -0.19 | 0.128 | 0.15 | 0.08 |
| C18:1 *cis*-12 | 17.7 | 0.79 | -6.57 | 3.597 | 0.08 | 0.11 |
| C18:1 *cis*-13 | 19.4 | 1.83 | -26.28 | 15.965 | 0.11 | 0.09 |
| C18:1 *trans*-6 | 18.1 | 1.04 | -7.69 | 4.405 | 0.09 | 0.10 |
| C18:1 *trans*-9 | 17.9 | 1.35 | -10.92 | 9.341 | 0.25 | 0.05 |
| C18:1 *trans*-10 | 17.4 | 0.63 | -4.34 | 2.411 | 0.08 | 0.11 |
| C18:1 *trans*-11 | 17.2 | 0.99 | -0.91 | 1.096 | 0.41 | 0.03 |
| C18:1 *trans*-15 + C18:1 *cis*-11 | 20.0 | 1.61 | -6.03 | 2.656 | 0.03 | 0.16 |
| C18:2 *cis*-9*, trans*-11 | 16.8 | 0.88 | -0.79 | 1.847 | 0.67 | < 0.01 |
| C18:2n-6 | 19.8 | 2.07 | -2.33 | 1.396 | 0.11 | 0.09 |
| C18:3n-3 | 14.7 | 1.17 | 4.24 | 2.829 | 0.15 | 0.08 |
| C18:3n-6 | 19.5 | 2.31 | -41.23 | 30.154 | 0.18 | 0.06 |
| C20:0 | 11.3 | 2.51 | 42.16 | 20.397 | 0.05 | 0.14 |
| C20:1 *cis*-11 | 18.8 | 1.74 | -51.95 | 37.519 | 0.18 | 0.07 |
| C20:2n-6 | 13.8 | 2.59 | 55.87 | 54.712 | 0.32 | 0.04 |
| C20:3n-6 | 16.6 | 1.88 | -2.60 | 20.529 | 0.90 | < 0.01 |
| C20:4n-3 | 16.4 | 0.75 | 0.76 | 9.405 | 0.94 | < 0.01 |
| C20:4n-6 | 14.8 | 1.96 | 12.36 | 14.720 | 0.41 | 0.03 |
| C20:5n-3 | 13.6 | 1.59 | 46.71 | 25.765 | 0.08 | 0.11 |
| C22:0 | 13.7 | 0.90 | 43.02 | 13.659 | < 0.01 | 0.27 |
| C22:5n-3 | 11.4 | 2.12 | 55.20 | 22.924 | 0.02 | 0.18 |
| C24:0 | 14.0 | 0.81 | 53.23 | 16.253 | < 0.01 | 0.28 |
| A Fat- and protein-corrected milk (kg/d) = [0.337 + 0.116 × fat (g/100 g milk) + 0.06 × protein (g/100 g milk)] × milk yield (kg/d) (CVB, 2012). | | | | | | |
| B C18:1 cis-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible, as this FA is always present in small amounts. | | | | | | |