Supplementary File – for Online Publication Only

**Supplementary Table S1** *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on milk 16:1 composition (g/100 g fatty acids) in lactating cows*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Experiment 1 | | | | | |  | Experiment 2 | | | | | |
|  | Treatment1 | | |  | *P*3 | |  | Treatment2 | | |  | *P*3 | |
| Fatty acid | HPO | HPS | HSO | s.e.m. | L | Q |  | HSO | MSO | LSO | s.e.m. | L | Q |
| *cis*-9 16:14 | 2.24 | 1.74 | 1.50 | 0.074 | <0.001 | 0.16 |  | 1.72 | 1.47 | 1.50 | 0.07 | 0.029 | 0.11 |
| *cis*-10 16:1 | 0.017 | 0.026 | 0.044 | 0.0038 | <0.001 | 0.29 |  | 0.053 | 0.042 | 0.051 | 0.0053 | 0.83 | 0.15 |
| *cis*-11 16:15 | 0.049 | 0.041 | 0.047 | 0.0034 | 0.57 | 0.09 |  | 0.061 | 0.045 | 0.053 | 0.0044 | 0.17 | 0.043 |
| *cis*-12 16:1 | 0.015 | 0.020 | 0.027 | 0.0013 | <0.001 | 0.40 |  | 0.029 | 0.027 | 0.026 | 0.0016 | 0.30 | 0.74 |
| *cis*-13 16:1 | 0.168 | 0.079 | 0.085 | 0.0080 | <0.001 | <0.001 |  | 0.056 | 0.067 | 0.068 | 0.0059 | 0.17 | 0.52 |
| *trans-*6+7 16:1 | 0.038 | 0.045 | 0.052 | 0.0014 | <0.001 | 0.95 |  | 0.065 | 0.051 | 0.055 | 0.0032 | 0.028 | 0.029 |
| *trans*-8 16:1 | 0.010 | 0.018 | 0.032 | 0.0045 | 0.002 | 0.64 |  | 0.144 | 0.029 | 0.030 | 0.0035 | 0.031 | 0.19 |
| *trans*-9 16:1 | 0.034 | 0.052 | 0.112 | 0.0082 | <0.001 | 0.048 |  | 0.137 | 0.123 | 0.143 | 0.0020 | 0.83 | 0.51 |
| *trans*-10 16:1 | 0.006 | 0.015 | 0.030 | 0.0019 | <0.001 | 0.26 |  | 0.032 | 0.024 | 0.023 | 0.0022 | 0.011 | 0.19 |
| *trans*-11 16:1 | 0.007 | 0.015 | 0.023 | 0.0018 | <0.001 | 0.89 |  | 0.034 | 0.025 | 0.027 | 0.0034 | 0.18 | 0.26 |
| *trans*-12 16:1 | 0.179 | 0.174 | 0.187 | 0.0056 | 0.28 | 0.20 |  | 0.203 | 0.180 | 0.184 | 0.0077 | 0.09 | 0.18 |
| *trans*-13 16:1 | 0.012 | 0.019 | 0.031 | 0.0011 | <0.001 | 0.06 |  | 0.032 | 0.029 | 0.031 | 0.0014 | 0.66 | 0.11 |

1Treatments consisted of high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).

2Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39:61 (HSO), 44:56 (MSO) or 48:52 (LSO).

3Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).

4Co-elutes with 17:0 *anteiso*.

5Co-elutes with 3S,7R,11R,15-tetramethyl-16:0.

**Supplementary Table S2** *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on milk odd- and branched-chain fatty acid composition (mg/100 g fatty acids) in lactating cows*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Experiment 1 | | | | | |  | Experiment 2 | | | | | |
|  | Treatment1 | | |  | *P*-value3 | |  | Treatment2 | | |  | *P*-value3 | |
| Fatty acid | HPO | HPS | HSO | SEM | L | Q |  | HSO | MSO | LSO | SEM | L | Q |
| 13:0 *anteiso* | 11.1 | 10.6 | 8.84 | 0.63 | 0.019 | 0.45 |  | 11.0 | 11.1 | 11.0 | 0.45 | 0.91 | 0.82 |
| 13:0 *iso* | 25.2 | 28.6 | 24.8 | 1.93 | 0.86 | 0.14 |  | 23.9 | 27.3 | 28.7 | 1.66 | 0.06 | 0.63 |
| 14:0 *iso* | 93.6 | 127 | 97.4 | 10.2 | 0.80 | 0.021 |  | 75.9 | 96.3 | 105 | 5.26 | <0.001 | 0.38 |
| 15:0 | 1384 | 1053 | 1039 | 57.9 | <0.001 | 0.033 |  | 1024 | 965 | 980 | 30.4 | 0.32 | 0.33 |
| 15:0 *anteiso* | 553 | 547 | 520 | 16.5 | 0.18 | 0.63 |  | 517 | 533 | 539 | 18.2 | 0.41 | 0.81 |
| 15:0 *iso* | 180 | 190 | 172 | 6.36 | 0.36 | 0.08 |  | 150 | 178 | 188 | 7.61 | 0.002 | 0.32 |
| *cis*-9 15:1 | 15.2 | 9.89 | 9.39 | 0.90 | <0.001 | 0.039 |  | 11.6 | 8.55 | 10.3 | 1.05 | 0.39 | 0.07 |
| *trans*-5 15:1 | 52.2 | 53.5 | 51.6 | 2.24 | 0.84 | 0.56 |  | 52.5 | 49.5 | 54.8 | 2.33 | 0.50 | 0.16 |
| *trans*-6 15:1 | 10.4 | 10.7 | 12.5 | 0.81 | 0.09 | 0.45 |  | 12.9 | 10.1 | 11.0 | 0.87 | 0.13 | 0.10 |
| *trans*-9 15:1 | 2.61 | 4.65 | 5.77 | 0.576 | <0.001 | 0.52 |  | 5.58 | 5.24 | 5.97 | 0.755 | 0.72 | 0.57 |
| *trans*-10 15:1 | 6.80 | 4.69 | 5.53 | 0.717 | 0.23 | 0.11 |  | 5.76 | 3.81 | 4.11 | 0.745 | 0.13 | 0.23 |
| 16:0 *iso* | 236 | 283 | 259 | 20.6 | 0.44 | 0.18 |  | 238 | 249 | 264 | 14.9 | 0.23 | 0.91 |
| 17:0 | 578 | 502 | 506 | 22.1 | 0.031 | 0.15 |  | 529 | 496 | 495 | 16.9 | 0.17 | 0.47 |
| 17:0 *iso* | 311 | 315 | 333 | 9.69 | 0.13 | 0.54 |  | 351 | 338 | 353 | 10.7 | 0.91 | 0.30 |
| *cis*-6+7 17:1 | 30.9 | 20.6 | 16.9 | 2.09 | <0.001 | 0.21 |  | 16.6 | 14.3 | 15.6 | 0.84 | 0.41 | 0.10 |
| *cis*-8 17:1 | 12.5 | 10.3 | 10.9 | 1.77 | 0.53 | 0.51 |  | 13.2 | 10.2 | 10.2 | 0.88 | 0.025 | 0.19 |
| *cis*-9 17:1 | 214 | 162 | 156 | 8.94 | <0.001 | 0.045 |  | 200 | 157 | 160 | 12.8 | 0.039 | 0.16 |
| 18:0 *iso* | 48.3 | 53.0 | 52.9 | 4.82 | 0.51 | 0.69 |  | 61.3 | 52.0 | 48.4 | 3.15 | 0.008 | 0.45 |
| 11-cyclohexyl 11:0 | 96.6 | 78.6 | 80.5 | 4.35 | 0.016 | 0.08 |  | 79.7 | 80.6 | 74.3 | 5.47 | 0.49 | 0.60 |
| 19:0 | 77.1 | 81.7 | 79.5 | 7.04 | 0.81 | 0.70 |  | 78.3 | 94.7 | 102 | 8.06 | 0.05 | 0.64 |
| *cis*-7 19:1 | 17.6 | 14.9 | 16.6 | 1.78 | 0.72 | 0.33 |  | 21.4 | 18.2 | 18.9 | 1.84 | 0.35 | 0.38 |
| *cis*-10 19:1 | 24.2 | 17.8 | 19.2 | 1.26 | 0.010 | 0.019 |  | 18.7 | 16.7 | 16.8 | 1.38 | 0.34 | 0.52 |
| 21:0 | 27.8 | 28.3 | 27.6 | 3.07 | 0.97 | 0.89 |  | 27.4 | 32.2 | 35.1 | 3.30 | 0.11 | 0.82 |
| 23:0 | 26.2 | 15.8 | 13.0 | 3.26 | 0.009 | 0.35 |  | 12.1 | 16.8 | 13.2 | 2.33 | 0.74 | 0.15 |
| 29:0 | 15.9 | 15.2 | 14.6 | 1.11 | 0.40 | 0.99 |  | 10.2 | 12.3 | 16.0 | 0.93 | <0.001 | 0.50 |

1Treatments consisted of high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).

2Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39:61 (HSO), 44:56 (MSO) or 48:52 (LSO).

3Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).



**Supplementary Figure S1** Correlation loading plot of Factor-2 versus Factor-1 after recalculation with 65 different fatty acids in milk as significant (*P* < 0.05) X-variables, determined by jack-knifing, which contribute to the explained variance of the Y-variables (MFP and MFY represent milk fat content and yield, respectively). Abbreviations *c* and *t* designate *cis* and *trans* positional isomers, respectively. Explained variances of X and Y are given for the respective factor in parenthesis on the axis. Factor axes are scaled according to the score coordinates in the bilinear model.



**Supplementary Figure S2** Distribution of 48 milk samples according to milk fat composition from cows fed high-concentrate diets containing 30 g/kg (on a dry matter basis) of supplemental fat as prilled palm fat (HPO), sunflower oil (HSO-1) or an equal mixture of both fat supplements (HPS) in experiment 1, or fed diets containing 30 g/kg sunflower oil with forage-to-concentrate ratio (on a dry matter basis) of 39:61 (HSO-2), 44:56 (MSO) or 48:52 (LSO) in experiment 2, in the score plot of Factor-2 versus Factor-1 for the partial least square regression model with milk fat content and yield as response variables. Explained variances of X and Y are given for the respective factor in parenthesis on the axis. Factor axes are scaled according to the score coordinates in the bilinear model.