

**Effect of increased milking frequency and residual milk removal on milk production and milk fatty acid composition in lactating cows**

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**SUPPLEMENTARY FILE**

**Supplementary Table S1.** Effect of two (2x) or four (4x) times daily milking combined without or with residual milk removal (RMR) on milk C16:1 and C16:2 concentrations

| g/100 g fatty acids                    | Available milk          |        |        |        |         | Residual milk         |              |              |                         |        | Available vs residual |        |         |                       |              |              |                       |              |              |              |
|--|-------------------------|--------|--------|--------|---------|-----------------------|--------------|--------------|-------------------------|--------|-----------------------|--------|---------|-----------------------|--------------|--------------|-----------------------|--------------|--------------|--------------|
|  | Treatments <sup>1</sup> |        |        |        | SEM     | P-values <sup>2</sup> |              |              | Treatments <sup>1</sup> |        |                       |        | SEM     | P-values <sup>2</sup> |              |              | P-values <sup>3</sup> |              |              |              |
|  | 2x                      | 2xRMR  | 4x     | 4xRMR  |         | MF                    | RMR          | MRxRMR       | 2x                      | 2xRMR  | 4x                    | 4xRMR  |         | MF                    | RMR          | MFxRMR       | 2x                    | 2xRMR        | 4x           | 4xRMR        |
| <i>trans</i> -4 16:1                   | 0.0033                  | 0.0043 | 0.0020 | 0.0043 | 0.00106 | 0.568                 | 0.158        | 0.568        | 0.0053                  | 0.0040 | 0.0040                | 0.0033 | 0.00079 | 0.246                 | 0.246        | 0.759        | 0.320                 | 0.718        | 0.201        | 0.546        |
| <i>trans</i> -5 16:1                   | 0.0045                  | 0.0075 | 0.0048 | 0.0075 | 0.00176 | 0.941                 | 0.124        | 0.941        | 0.0273                  | 0.0065 | 0.0133                | 0.0058 | 0.00810 | 0.344                 | 0.097        | <b>0.039</b> | 0.206                 | 0.530        | 0.407        | 0.614        |
| <i>trans</i> -6-8 16:1                 | 0.047                   | 0.053  | 0.045  | 0.049  | 0.0036  | 0.383                 | 0.181        | 0.725        | 0.043                   | 0.048  | 0.045                 | 0.051  | 0.0027  | 0.256                 | <b>0.033</b> | 0.810        | 0.519                 | 0.423        | 0.976        | 0.593        |
| <i>trans</i> -9 16:1                   | 0.048                   | 0.058  | 0.051  | 0.053  | 0.0033  | 0.621                 | <b>0.046</b> | 0.169        | 0.046                   | 0.548  | 0.050                 | 0.055  | 0.0028  | 0.240                 | <b>0.007</b> | 0.350        | 0.693                 | 0.182        | 0.731        | 0.529        |
| <i>trans</i> -12 16:1                  | 0.019                   | 0.021  | 0.019  | 0.018  | 0.0026  | 0.440                 | 0.818        | 0.552        | 0.028                   | 0.023  | 0.029                 | 0.022  | 0.0029  | 0.934                 | 0.073        | 0.679        | <b>0.011</b>          | 0.581        | <b>0.040</b> | 0.235        |
| <i>trans</i> -13 16:1                  | 0.018                   | 0.019  | 0.017  | 0.017  | 0.0017  | 0.376                 | 0.625        | 0.833        | 0.018                   | 0.017  | 0.017                 | 0.018  | 0.0030  | 0.925                 | 0.925        | 0.778        | 0.874                 | 0.439        | 0.953        | 0.950        |
| <i>cis</i> -9 16:1                     | 1.17                    | 1.15   | 1.07   | 1.24   | 0.095   | 0.825                 | <b>0.023</b> | <b>0.006</b> | 1.17                    | 1.12   | 1.06                  | 1.116  | 0.10200 | 0.119                 | 0.976        | 0.137        | 0.999                 | 0.094        | 0.970        | <b>0.032</b> |
| <i>cis</i> -11 16:1                    | 0.025                   | 0.030  | 0.025  | 0.031  | 0.0029  | 0.885                 | <b>0.019</b> | 0.772        | 0.025                   | 0.028  | 0.027                 | 0.026  | 0.0028  | 0.702                 | 0.273        | 0.063        | 0.866                 | 0.509        | 0.595        | 0.360        |
| <i>cis</i> -15 16:1                    | 0.24                    | 0.28   | 0.23   | 0.30   | 0.037   | 0.756                 | 0.081        | 0.519        | 0.22                    | 0.21   | 0.22                  | 0.28   | 0.027   | 0.062                 | 0.134        | 0.066        | 0.390                 | <b>0.031</b> | 0.818        | 0.394        |
| <i>trans</i> -9, <i>trans</i> -13 16:2 | 0.0093                  | 0.0038 | 0.0088 | 0.0085 | 0.00123 | 0.091                 | <b>0.035</b> | <b>0.048</b> | 0.0063                  | 0.0070 | 0.0100                | 0.0060 | 0.00178 | 0.426                 | 0.352        | 0.191        | 0.302                 | 0.168        | 0.431        | 0.368        |
| <i>cis</i> -9, <i>cis</i> -12 16:2     | 0.0033                  | 0.0015 | 0.0035 | 0.0018 | 0.00044 | 0.585                 | <b>0.003</b> | 1.000        | 0.0033                  | 0.0038 | 0.0043                | 0.0020 | 0.00080 | 0.639                 | 0.294        | 0.121        | 1.000                 | <b>0.018</b> | 0.391        | 0.789        |

<sup>1</sup>Values represent least square means of measurements made for samples collected from four cows at the end of a 96 h treatment period.

<sup>2</sup>Probability of effects due to milking frequency (MF), residual milk removal (RMR) and their interaction (MF×RMR). Bold typeface indicates significant differences ( $P \leq 0.05$ )

<sup>3</sup>Probability of differences between available and residual milk collected on the last milking during each treatment period. Bold typeface indicates significant differences ( $P \leq 0.05$ ).

**Supplementary Table S2.** Effect of two (2x) or four (4x) times daily milking combined without or with residual milk removal (RMR) on milk odd and branched chain fatty acid concentrations

| g/100 g fatty acids   | Available milk          |        |                       |        |         | Residual milk           |              |                       |        |        | Available vs residual |        |         |              |              |              |              |              |       |              |
|---|-------------------------|--------|-----------------------|--------|---------|-------------------------|--------------|-----------------------|--------|--------|-----------------------|--------|---------|--------------|--------------|--------------|--------------|--------------|-------|--------------|
|   | Treatments <sup>1</sup> |        | P-values <sup>2</sup> |        |         | Treatments <sup>1</sup> |              | P-values <sup>2</sup> |        |        | P-values <sup>3</sup> |        |         |              |              |              |              |              |       |              |
|   | 2x                      | 2xRMR  | 4x                    | 4xRMR  | SEM     | MF                      | RMR          | MFxRMR                | 2x     | 2xRMR  | 4x                    | 4xRMR  | SEM     | MF           | RMR          | MFxRMR       | 2x           | 2xRMR        | 4x    | 4xRMR        |
| 5:0   | 0.029                   | 0.027  | 0.030                 | 0.030  | 0.0014  | 0.113                   | 0.559        | 0.457                 | 0.032  | 0.028  | 0.032                 | 0.029  | 0.0021  | 0.813        | 0.134        | 0.638        | 0.072        | 0.194        | 0.534 | 0.092        |
| 7:0   | 0.031                   | 0.023  | 0.030                 | 0.029  | 0.0025  | 0.291                   | 0.112        | 0.195                 | 0.032  | 0.025  | 0.033                 | 0.028  | 0.0025  | 0.508        | <b>0.050</b> | 0.634        | 0.722        | 0.096        | 0.426 | 0.353        |
| 9:0   | 0.043                   | 0.031  | 0.042                 | 0.042  | 0.0037  | 0.213                   | 0.147        | 0.161                 | 0.043  | 0.034  | 0.044                 | 0.039  | 0.0040  | 0.471        | 0.092        | 0.671        | 0.919        | <b>0.032</b> | 0.646 | 0.164        |
| 11:0  | 0.076                   | 0.055  | 0.076                 | 0.076  | 0.0074  | 0.218                   | 0.218        | 0.208                 | 0.076  | 0.058  | 0.079                 | 0.071  | 0.0077  | 0.326        | 0.120        | 0.532        | 0.980        | <b>0.035</b> | 0.736 | 0.461        |
| 13:0  | 0.10                    | 0.09   | 0.11                  | 0.11   | 0.008   | 0.279                   | 0.355        | 0.430                 | 0.10   | 0.09   | 0.11                  | 0.10   | 0.008   | 0.273        | 0.216        | 0.811        | 0.814        | 0.718        | 0.899 | 0.311        |
| iso13:0   | 0.020                   | 0.022  | 0.019                 | 0.018  | 0.0025  | 0.132                   | 0.812        | 0.396                 | 0.019  | 0.021  | 0.018                 | 0.019  | 0.0025  | 0.287        | 0.510        | 0.605        | 0.787        | 0.215        | 0.872 | 0.911        |
| anteiso13:0   | 0.0073                  | 0.0095 | 0.0073                | 0.0075 | 0.00089 | 0.302                   | 0.208        | 0.302                 | 0.0063 | 0.0080 | 0.0068                | 0.0073 | 0.00079 | 0.878        | 0.189        | 0.450        | 0.423        | 0.103        | 0.718 | 0.824        |
| cis-9 13:1  | 0.0078                  | 0.0058 | 0.0073                | 0.0080 | 0.00122 | 0.440                   | 0.576        | 0.242                 | 0.0060 | 0.0053 | 0.0065                | 0.0060 | 0.00123 | 0.580        | 0.580        | 0.911        | 0.340        | 0.495        | 0.761 | 0.295        |
| iso14:0   | 0.080                   | 0.088  | 0.083                 | 0.077  | 0.0095  | 0.408                   | 0.777        | 0.136                 | 0.077  | 0.087  | 0.081                 | 0.082  | 0.0098  | 0.979        | 0.272        | 0.363        | 0.852        | 0.375        | 0.893 | 0.662        |
| 15:0  | 1.03                    | 1.02   | 1.03                  | 1.03   | 0.029   | 0.691                   | 0.755        | 1.000                 | 1.01   | 1.01   | 1.03                  | 1.00   | 0.030   | 0.764        | 0.529        | 0.491        | 0.758        | 0.128        | 0.953 | 0.240        |
| iso15:0   | 0.19                    | 0.21   | 0.19                  | 0.18   | 0.017   | 0.086                   | 0.448        | <b>0.034</b>          | 0.18   | 0.20   | 0.19                  | 0.19   | 0.019   | 0.508        | 0.161        | 0.106        | 0.821        | <b>0.008</b> | 0.909 | 0.769        |
| anteiso15:0   | 0.42                    | 0.41   | 0.43                  | 0.40   | 0.024   | 0.895                   | 0.244        | 0.418                 | 0.40   | 0.40   | 0.42                  | 0.42   | 0.024   | 0.387        | 0.944        | 0.944        | 0.687        | 0.106        | 0.340 | 0.098        |
| R2,R6,R10,14-tetramethyl-15:0                                 | 0.0070                  | 0.0195 | 0.0095                | 0.0180 | 0.00409 | 0.880                   | <b>0.016</b> | 0.551                 | 0.0040 | 0.0060 | 0.0050                | 0.0143 | 0.00190 | <b>0.020</b> | <b>0.009</b> | <b>0.050</b> | 0.387        | <b>0.024</b> | 0.522 | 0.499        |
| trans-5 15:1  | 0.052                   | 0.049  | 0.051                 | 0.047  | 0.0028  | 0.313                   | 0.087        | 0.764                 | 0.052  | 0.050  | 0.051                 | 0.052  | 0.0040  | 0.939        | 0.819        | 0.596        | 0.927        | 0.591        | 0.964 | 0.285        |
| trans-6 15:1  | 0.028                   | 0.027  | 0.027                 | 0.024  | 0.0024  | 0.151                   | 0.211        | 0.510                 | 0.028  | 0.028  | 0.027                 | 0.026  | 0.0028  | 0.390        | 0.660        | 0.513        | 1.000        | 0.252        | 0.671 | <b>0.035</b> |
| cis-9 15:1  | 0.012                   | 0.015  | 0.011                 | 0.014  | 0.0027  | 0.531                   | 0.093        | 0.832                 | 0.010  | 0.010  | 0.010                 | 0.014  | 0.0015  | <b>0.005</b> | <b>0.017</b> | <b>0.005</b> | 0.465        | 0.109        | 0.960 | 0.813        |
| iso16:0   | 0.18                    | 0.20   | 0.20                  | 0.18   | 0.020   | 0.826                   | 0.907        | 0.061                 | 0.18   | 0.19   | 0.19                  | 0.19   | 0.022   | 0.597        | 0.483        | 0.302        | 0.936        | 0.059        | 0.811 | 0.562        |
| 17:0  | 0.39                    | 0.41   | 0.41                  | 0.40   | 0.017   | 0.708                   | 0.952        | 0.398                 | 0.39   | 0.41   | 0.41                  | 0.40   | 0.016   | 0.467        | 0.664        | 0.251        | 0.918        | 0.798        | 0.977 | 0.916        |
| iso17:0   | 0.38                    | 0.40   | 0.39                  | 0.37   | 0.010   | 0.226                   | 0.813        | <b>0.034</b>          | 0.38   | 0.40   | 0.39                  | 0.39   | 0.008   | 0.923        | 0.307        | 0.246        | 0.935        | 0.358        | 0.899 | 0.250        |
| anteiso17:0   | 0.36                    | 0.36   | 0.36                  | 0.34   | 0.024   | 0.733                   | 0.786        | 0.777                 | 0.34   | 0.35   | 0.36                  | 0.36   | 0.022   | 0.608        | 0.752        | 0.777        | 0.569        | 0.133        | 0.981 | 0.108        |
| S3,R7,R11,15-tetramethyl-16:0 + R3,R7,R11,15-tetramethyl-16:0 | 0.10                    | 0.10   | 0.12                  | 0.91   | 0.032   | 0.907                   | 0.608        | 0.608                 | 0.06   | 0.08   | 0.07                  | 0.06   | 0.011   | 0.561        | 0.414        | 0.211        | 0.379        | 0.481        | 0.329 | 0.233        |
| trans-6 17:1  | 0.015                   | 0.012  | 0.014                 | 0.014  | 0.0007  | 0.271                   | <b>0.020</b> | 0.072                 | 0.014  | 0.014  | 0.014                 | 0.016  | 0.0013  | 0.356        | 0.482        | 0.635        | 0.547        | 0.078        | 0.926 | 0.456        |
| cis-9 17:1  | 0.12                    | 0.13   | 0.12                  | 0.12   | 0.006   | 0.109                   | 0.655        | 1.000                 | 0.12   | 0.12   | 0.12                  | 0.12   | 0.006   | 0.115        | 0.818        | 0.453        | 0.891        | 0.854        | 0.937 | 0.359        |
| iso18:0   | 0.10                    | 0.11   | 0.10                  | 0.09   | 0.009   | 0.177                   | 0.839        | 0.104                 | 0.10   | 0.11   | 0.10                  | 0.10   | 0.008   | 0.130        | 0.476        | 0.078        | 0.944        | 0.468        | 0.955 | 0.082        |
| cis-9 19:1  | 0.084                   | 0.083  | 0.079                 | 0.085  | 0.0062  | 0.788                   | 0.666        | 0.581                 | 0.077  | 0.076  | 0.081                 | 0.083  | 0.0040  | 0.068        | 0.814        | 0.545        | 0.429        | 0.158        | 0.851 | 0.669        |
| cis-11 19:1   | 0.013                   | 0.010  | 0.015                 | 0.009  | 0.0033  | 0.723                   | 0.166        | 0.598                 | 0.015  | 0.012  | 0.016                 | 0.007  | 0.0032  | 0.651        | 0.094        | 0.373        | 0.638        | 0.485        | 0.843 | 0.408        |
| cis-12 19:1   | 0.007                   | 0.005  | 0.004                 | 0.003  | 0.0016  | 0.121                   | 0.335        | 0.651                 | 0.002  | 0.005  | 0.003                 | 0.006  | 0.0008  | 0.074        | <b>0.001</b> | 0.680        | 0.086        | 0.928        | 0.690 | 0.266        |
| 23:0  | 0.038                   | 0.021  | 0.038                 | 0.024  | 0.0062  | 0.754                   | <b>0.032</b> | 0.814                 | 0.059  | 0.034  | 0.056                 | 0.045  | 0.0095  | 0.691        | 0.099        | 0.443        | <b>0.011</b> | 0.069        | 0.224 | 0.344        |
| cis-14 23:1   | 0.043                   | 0.047  | 0.041                 | 0.036  | 0.0101  | 0.295                   | 0.983        | 0.431                 | 0.052  | 0.052  | 0.052                 | 0.040  | 0.0095  | 0.496        | 0.496        | 0.515        | 0.651        | 0.260        | 0.529 | 0.448        |
| 25:0  | 0.0115                  | 0.0168 | 0.0120                | 0.0080 | 0.00537 | 0.462                   | 0.910        | 0.412                 | 0.0050 | 0.0103 | 0.0120                | 0.0080 | 0.00362 | 0.493        | 0.854        | 0.205        | 0.294        | 0.389        | 1.000 | 1.000        |

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<sup>2</sup>Probability of effects due to milking frequency (MF), residual milk removal (RMR) and their interaction (MF×RMR). Bold typeface indicates significant differences ( $P \leq 0.05$ ).

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