Response profiles of dairy cows to a single 24h milking interval in relation with milk proteolysis, udder compliance and immune traits

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***Effects of a 24h*** ***milking interval on traits.***

Statistical analyses were performed using R software (RCore Team, The R Foundation for Statistical Computing, 2014, R: A Language and Environment for Statistical Computing, Version 3.0.2, Vienna, Austria, http:/www.R-project.org). The data were analyzed according to the following linear mixed model (1):



where  is dependent variable and µ is the mean, the fixed effects tested are period *i* (cTDM, 24h-MI), stage of lactation *j* (4 classes: 24-50, 51-100, 101-180, 181-311 DIM), parity *k* (3 classes: primiparous, 2nd lactation and 3rd-plus lactation), and the 2-way interaction stage of lactation  parity, random effect of the cow (assumed to be normally distributed) is *l* and is the residual error associated with each *ijkl* observation. The fixed effects other than period were coded as described in Charton et al. (2016). Results for fixed effects in models were expressed as least-squares means and computed using the lsmeans package ( Lenth R 2014. lsmeans: Least-Squares Means. R package version 2.12. Retrieved on 14 April 2015 from [http://CRAN.R-project.org/package=lsmeans](http://CRAN.R-project.org/package%3Dlsmeans).). Differences were considered significant at *P* < 0.05.

Table S1 Effects on traits of 24 h milking interval (24h-MI) compared to control-period twice-daily milking (cTDM) in dairy cows. Least-square means ± standard error of the mean (SEM) and number of observations per traits (n)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | cTDM | 24h-MI | SEM | Signif. | n | RMSE4 |
| Milk proteolysis |  |  |  |  |  |  |
| Milk plasmin activity, OD1 × 104/min | 10.7 | 12.9 | 0.90 | \*\*\* | 97 | 2.49 |
| Milk plasminogen, OD × 104/min | 156.3 | 169.2 | 3.80 | \*\*\* | 97 | 12.13 |
| Milk plasminogen:plasmin ratio, % | 19.7 | 19.4 | 1.75 | NS | 97 | 3.49 |
| “Peptide” mapping zone Z1, mAU2/min | 152.5 | 134.4 | 4.89 | \*\*\* | 97 | 17.03 |
| “Peptide” mapping zone Z2, mAU/min | 468.8 | 644.7 | 25.64 | \*\*\* | 97 | 74.76 |
| “Peptide” mapping zone Z3, mAU/min | 274.2 | 481.0 | 29.22 | \*\*\* | 97 | 95.89 |
| Dry matter intake |  |  |  |  |  |  |
| Total dry matter intake (kg) | 22.2 | 21.6 | 0.23 | \*\*\* | 202 | 1.50 |
| Udder distention |  |  |  |  |  |  |
| Left udder side teats distance, cm | 13.9 | 15.9 | 0.35 | \*\*\* | 97 | 0.84 |
| Front udder side teats distance, cm | 15.4 | 19.2 | 0.58 | \*\*\* | 97 | 1.10 |
| Right udder side teats distance, cm | 13.7 | 15.5 | 0.34 | \*\*\* | 97 | 0.92 |
| Rear udder side teats distance, cm | 5.4 | 9.5 | 0.42 | \*\*\* | 97 | 1.06 |
| Sum of udder teat distances, cm | 48.4 | 60.0 | 1.27 | \*\*\* | 97 | 2.74 |
| Milking parameters |  |  |  |  |  |  |
| Milking duration3, min | 6.3 | 10.4 | 0.19 | \*\*\* | 435 | 1.47 |
| Average milk flow rate3, kg/min | 2.0 | 2.5 | 0.04 | \*\*\* | 435 | 0.27 |
| Peak milking milk flow rate3, kg/min | 3.4 | 3.6 | 0.06 | \*\*\* | 435 | 0.28 |
| Amount of milk collected at 1 min3, kg | 1.1 | 1.9 | 0.04 | \*\*\* | 435 | 0.35 |
| Amount of milk collected at 2 min3, kg | 3.6 | 5.0 | 0.10 | \*\*\* | 435 | 0.68 |
| Proportion of milk collected at 1 min3, % | 9 | 8 | 0.3 | \*\*\* | 435 | 2.2 |
| Proportion of milk collected at 2 min3, % | 30 | 21 | 0.7 | \*\*\* | 435 | 5.0 |
| Mammary epithelium permeability |  |  |  |  |  |  |
| Blood plasma lactose (mg/L) | 28.82 | 172.40 | 6.005 | \*\*\* | 721 | 124.28 |
| Log-transformed blood lactose (log, units) | 4.36 | 6.76 | 0.043 | \*\*\* | 721 | 0.91 |
| Systemic inflammation |  |  |  |  |  |  |
| Blood plasma haptoglobin, mg/mL | 0.42 | 0.34 | 0.041 | \* | 97 | 0.211 |
| Complete Blood Count (CBC) |  |  |  |  |  |  |
| Red blood cells (RBCs), × 106/mm3 | 6.15 | 6.20 | 0.107 | NS | 65 | 0.213 |
| Hematocrit, % | 29.52 | 29.61 | 0.477 | NS | 65 | 1.159 |
| Haemoglobin (Hg), g/dL | 10.53 | 10.30 | 0.146 | \* | 65 | 0.333 |
| Mean corpuscular Hg conc. (MCHC), g/mL | 35.81 | 34.83 | 0.158 | \*\*\* | 65 | 0.797 |
| Mean corpuscular volume (MCV), µm3 | 48 | 48 | 0.6 | NS | 65 | 0.6 |
| Blood platelets, × 103/mm3 | 286 | 341 | 24.1 | \*\* | 65 | 61.9 |
| White blood cells (WBCs), × 103/mm3 | 9.67 | 9.65 | 0.556 | NS | 65 | 1.624 |
| Polymorphonuclear neutrophils, % | 46 | 45 | 2.5 | NS | 65 | 9.2 |
| Polymorphonuclear eosinophils, % | 4 | 5 | 0.7 | NS | 65 | 2.3 |
| Polymorphonuclear basophils, % | 0.3 | 0.4 | 0.14 | NS | 65 | 0.71 |
| Lymphocytes, % | 44 | 47 | 2.3 | NS | 65 | 9.2 |
| Monocytes, % | 6 | 3 | 0.5 | \*\*\* | 65 | 2.6 |

1 OD=optical density.

2 mAU = milli absorption units defined as 1 AU/1000.

3 measure made during the afternoon milking.

\*\*\*, \*\*, \*, NS: significantly different from 0 at *P* < 0.001, *P* < 0.01, *P* < 0.05 and non-significant at *P* < 0.05, respectively.

4Root mean square error.