**SUPPLEMENTAL MATERIALS OF FLOW-RESPONSIVE PULSATION**

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ANALYTICAL APPROACH

## *Teat Tissue Condition*

To determine differences in STC between groups, a generalized linear mixed model with a logit link and a binomial distribution was fitted with PROC GLIMMIX. Treatment and period nested with treatment were forced into the model as fixed effects. The lactation number (1st, 2nd, or ≥3rd lactation), stage of lactation (≤ 100, 101–200, or > 200 DIM), and SCC (log10-transformed, logSCC) were considered additional independent variables. The random effects of cows and cows crossed within a period were considered to account for the variabilities within the cows and treatment periods. Collinearity among eligible variables was assessed by calculating Spearman correlation coefficients in PROC CORR. A coefficient of > |0.50| was considered to indicate collinearity. No collinearity was observed (| r | ≤ 0.03). Manual backward elimination was performed until each of the variables had a *P-*value ≤ 0.05 to establish the final model. Tukey–Kramer post hoc tests were used for multiple comparisons.

## *Milking Characteristics*

For the analysis of milking characteristics, we excluded the first 2 d of each period. Consequently, each period consisted of a 2-d adjustment and a 5-d data collection period. The observations with missing values, outliers or probable data errors were removed by screening as described by Reinemann et al. (2021). Milking observations with a value of MY < 2.5 kg, AMF < 0.1 or > 6 kg/min, PMF < 0.2 or > 10 kg/min, or MUOT < 100 or > 800 s were eliminated from the analyses.

To study the effect of treatment on the milking characteristics (MY, MUOT, 2MIN, PMF and duration of low milk flow rate; LMF), 5 separate general linear mixed models were fitted with PROC MIXED. The following steps were followed for all the models. To account for the clustering of milking observations within the cow, milking session and treatment period, we included random effects for cows, cow crossed with session, and cow crossed with study period. Treatment, session, and period nested with treatment were forced into the model as fixed effects. The inclusion of additional independent variables in the model was performed as described above. No collinearity was observed among the eligible variables (| r | ≤ 0.08). Confounding was assessed by observing regression coefficient changes. Variables for which the regression coefficients were altered by > 20% were considered confounding factors. Tukey–Kramer post hoc tests were used for multiple comparisons. For all final models, the assumptions of homoscedasticity and normality of residuals were assessed by the inspection of residual plots versus corresponding predicted values and the examination of quantile‒quantile residual plots. To satisfy these assumptions, the values of the dependent variables MUOT and LMF were log-transformed. The LSM estimates were back transformed and are presented as the geometric mean and 95% CI.

## *Bimodality and Milking Irregularities*

To determine the effect of treatment on bimodality, milking liner slip, milking unit kick-off, and milking-unit reattachment, 4 separate generalized linear mixed models with a logit link and a binomial distribution were generated with PROC GLIMMIX. The models were constructed in accordance with the procedure outlined above for the effect of treatment on teat tissue changes, with the inclusion of 3 levels of milking sessions (sessions 1, 2, and 3) as covariates. Three covariance structures (compound symmetry, variance components and autoregressive order 1) were tested to model the covariance of repeated measurements, with cows considered as subjects to correct for clustering of data within cows. The structure that resulted in the smallest pseudo-Akaike information criterion was selected.

Each 3-wk study period consisted of a 2-d adjustment period and a 19-d data collection period. The data maintenance, processing, and analytical procedures for milking characteristics, milking irregularities, and vacuum measurements were in accordance with Trial I.

## *Teat-end Hyperkeratosis*

A generalized linear mixed model was fitted with PROC GLIMMIX using a logit link and a binomial distribution to determine differences in HK between the groups. The consideration of independent and random variables and the modeling process were in accordance with the procedure described for the analysis of STC. No collinearity was observed among the independent variables (| r | ≤ 0.07). Treatment and period nested with treatment were forced into the model. Manual backward elimination was performed until each of the variables had a *P-*value ≤ 0.05 to establish the final model.

## *Hindleg Activity*

Two separate generalized mixed models with a log link and negative binomial distribution were used with PROC GLIMMIX to model the stepping and kicking separately. The consideration of fixed and random effects and the subsequent steps of modeling were similar to those used to analyze STC. The LSM were obtained using the ‘lsmeans’ and ‘ilink’ options and are presented with 95% CI.

SUPPLEMENTAL TABLES

**Supplemental Table S1.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, and stage of lactation on machine milking-induced short term changes in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | <0.0001 |  |
| FRP | −0.5 (0.2) |  | 0.41(0.31–0.55) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.0002 |  |
| Period 2 (FRP) | 0.1 (0.2)c |  | 0.65 (0.38−1.13) |
| Period 4 (FRP) | Referentc |  | 0.58 (0.30−1.14) |
| Period 1 (CON) | 0.9 (0.2)a |  | 2.41 (1.39−4.18) |
| Period 3 (CON) | 0.3 (0.2)b |  | 1.38 (0.80−2.39) |
| Period 5 (CON) | Referentbc |  | − |
| Lactation number |  | 0.004 |  |
| 1st | 0.8 (0.3)a |  | 2.28 (1.37–3.78) |
| 2nd | 0.2 (0.3)b |  | 1.16 (0.67–2.01) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | 0.002 |  |
| ≤ 100 DIM | 0.9 (0.3)a |  | 2.46 (1.42–4.25) |
| 101–200 DIM | 0.7 (0.3)a |  | 2.06 (1.24–3.40) |
| > 200 DIM | Referentb |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc test.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S2.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, log10-transformed somatic cell count (logSCC), and milking session on milk yield (kg/session) in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.99 |  |
| FRP | 0.3 (0.1) |  | 14.3 (13.8–14.7) |
| CON | Referent |  | 14.3 (13.8–14.7) |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | 0.1 (0.1)b |  | 14.3 (13.9−14.8) |
| Period 4 (FRP) | Referentb |  | 14.3 (13.8−14.7) |
| Period 1 (CON) | 0.6 (0.1)a |  | 15.6 (14.1−15.0) |
| Period 3 (CON) | 0.4 (0.1)b |  | 14.3 (13.5−14.4) |
| Period 5 (CON) | Referentc |  | 14.0 (13.5−14.4) |
| Lactation number |  | <0.0001 |  |
| 1st | −3.6 (0.5)b |  | 12.1 (11.4–12.8) |
| 2nd | −0.7 (0.5)a |  | 15.0 (14.2–15.9) |
| ≥ 3rd | Referenta |  | 15.7 (15.0–16.4) |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 2.6 (0.5)a |  | 15.4 (14.6–16.3) |
| 101–200 DIM | 1.8 (0.5)a |  | 14.6 (13.9–15.4) |
| > 200 DIM | Referentb |  | 12.8 (12.1–13.4) |
| LogSCC | −0.8 (0.4) | 0.03 | – |
| Milking session |  | <0.0001 |  |
| Session 1 | 3.6 (0.1)a |  | 17.1 (16.6–17.5) |
| Session 2 | −1.1 (0.1)c |  | 12.3 (11.9–12.8) |
| Session 3 | Referentb |  | 13.5 (13.0–13.9) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S3.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session and log10-transformed somatic cell count (logSCC) on machine-on time (s) in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.04 |  |
| FRP | 0.01 (0.002) |  | 272 (264–281) |
| CON | Referent |  | 270 (262–278) |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | 0.003 (0.003)ab |  | 273 (265−282) |
| Period 4 (FRP) | Referentb |  | 272 (263−280) |
| Period 1 (CON) | 0.02 (0.003)a |  | 277 (268−285) |
| Period 3 (CON) | 0.01 (0.003)b |  | 271 (263−279) |
| Period 5 (CON) | Referentc |  | 263 (255−271) |
| Lactation number |  | <0.0001 |  |
| 1st | −0.09 (0.01)b |  | 240 (229–252) |
| 2nd | ­­−0.02 (0.02)a |  | 281 (266–298) |
| ≥ 3rd | Referenta |  | 295 (282–309) |
| Stage of lactation |  | 0.0002 |  |
| ≤ 100 DIM | 0.07 (0.02)a |  | 296 (280–314) |
| 101–200 DIM | 0.02 (0.01)b |  | 266 (253–279) |
| > 200 DIM | Referentb |  | 253 (242–264) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.06 (0.002)a |  | 303 (295–313) |
| Session 2 | −0.03 (0.002)c |  | 248 (240–255) |
| Session 3 | Referentb |  | 265 (258–273) |
| LogSCC | −0.05 (0.01) | <0.0001 | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S4.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, stage of lactation, milking session and log10-transformed somatic cell count (logSCC) on 2-minute milk yield (kg) in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| FRP | −0.2 (0.1) |  | 5.8 (5.5–6.1) |
| CON | Referent |  | 6.0 (5.7–6.3) |
| Period (Treatment) |  | 0.51 |  |
| Period 2 (FRP) | −0.04 (0.06) |  | 5.8 (5.5−6.1) |
| Period 4 (FRP) | Referent |  | 5.8 (5.5−6.1) |
| Period 1 (CON) | 0.04 (0.06) |  | 6.0 (5.7−6.3) |
| Period 3 (CON) | −0.03 (0.06) |  | 6.0 (5.7−6.3) |
| Period 5 (CON) | Referent |  | 6.0 (5.7−6.3) |
| Stage of lactation |  | 0.05 |  |
| ≤ 100 DIM | 0.2 (0.4)ab |  | 5.8 (5.2–6.4) |
| 101–200 DIM | 0.8 (0.3)a |  | 6.4 (5.9–6.9) |
| > 200 DIM | Referentb |  | 5.6 (5.1–6.0) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.4 (0.1)a |  | 6.3 (6.0–6.6) |
| Session 2 | −0.5 (0.1)c |  | 5.4 (5.1–5.7) |
| Session 3 | Referentb |  | 5.9 (5.6–6.2) |
| LogSCC | 0.7 (0.3) | 0.005 | − |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S5.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, milking session and log10-transformed somatic cell count (logSCC) on peak milk flow rate (kg/min) in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.005 |  |
| FRP | 0.06 (0.03) |  | 5.0 (4.8–5.1) |
| CON | Referent |  | 4.9 (4.7–5.1) |
| Period (Treatment) |  | 0.02 |  |
| Period 2 (FRP) | −0.07 (0.03)ab |  | 4.9 (4.7−5.1) |
| Period 4 (FRP) | Referenta |  | 5.0 (4.8−5.2) |
| Period 1 (CON) | −0.05 (0.03)b |  | 4.9 (4.7−5.1) |
| Period 3 (CON) | −0.01 (0.03)b |  | 4.9 (4.7−5.1) |
| Period 5 (CON) | Referentab |  | 4.9 (4.8−5.1) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.1 (0.02)a |  | 5.1 (4.9–5.2) |
| Session 2 | −0.1 (0.02)c |  | 4.8 (4.7–5.0) |
| Session 3 | Referentb |  | 4.9 (4.7–5.1) |
| LogSCC | 0.4 (0.2) | 0.01 | − |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S6.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and the interaction between treatment and stage of lactation on duration of low milk flow rate (s) in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| FRP | 0.1 (0.01) |  | – |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.003 |  |
| Period 2 (FRP) | 0.006 (0.01)a |  | 22 (21−24) |
| Period 4 (FRP) | Referenta |  | 22 (21−24) |
| Period 1 (CON) | −0.02 (0.01)c |  | 17 (16−18) |
| Period 3 (CON) | 0.01 (0.01)b |  | 18 (17−19) |
| Period 5 (CON) | Referentbc |  | 18 (16−19) |
| Lactation number |  | 0.04 |  |
| 1st | 0.06 (0.03)ab |  | 20 (18–22) |
| 2nd | 0.08 (0.03)a |  | 21 (19–24) |
| ≥ 3rd | Referentb |  | 18 (16–19) |
| Stage of lactation |  | 0.02 |  |
| ≤ 100 DIM | −0.08 (0.03)ab |  | − |
| 101–200 DIM | −0.10 (0.03)b |  | − |
| > 200 DIM | Referenta |  | − |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.14 (0.01)b |  | 16 (15–17) |
| Session 2 | 0.001 (0.01)a |  | 22 (20–23) |
| Session 3 | Referenta |  | 22 (20–23) |
| Treatment × DIM |  | 0.01 |  |
| FRP × ≤ 100 DIM | 0.04 (0.01)ab |  | 22 (19–25) |
| CON × ≤ 100 DIM | Referentcd |  | 17 (15–19) |
| FRP × 101–200 DIM | 0.03 (0.01)abc |  | 21 (18–23) |
| CON × 101–200 DIM | Referentd |  | 16 (14–18) |
| FRP × > 200 DIM | Referenta |  | 24 (22–27) |
| CON × > 200 DIM | Referentbc |  | 20 (18–22) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S7.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and log10-transformed somatic cell count (LogSCC) on bimodality in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | <0.0001 |  |
| FRP | −0.5 (0.1) |  | 0.67 (0.61–0.74) |
| CON | Referent |  | – |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | −0.1 (0.1)a |  | 0.50 (0.41−0.62) |
| Period 4 (FRP) | Referentab |  | 0.58 (0.47−0.72) |
| Period 1 (CON) | −0.4 (0.1)bc |  | 0.66 (0.54−0.81) |
| Period 3 (CON) | −0.2 (0.1)c |  | 0.79 (0.64−0.97) |
| Period 5 (CON) | Referentd |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | −0.3 (0.1)c |  | 0.78 (0.69–0.88) |
| 2nd | 0.6 (0.1)a |  | 1.78 (1.59–2.00) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | −1.5 (0.1)c |  | 0.23 (0.20–0.26) |
| 101–200 DIM | −0.6 (0.1)b |  | 0.52 (0.47–0.58) |
| > 200 DIM | Referenta |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.1 (0.1)b |  | 0.88 (0.78–1.00) |
| Session 2 | 0.8 (0.1)a |  | 2.31 (2.05–2.59) |
| Session 3 | Referentb |  | – |
| LogSCC | 0.5 (0.04) | <0.0001 | 1.63 (1.50–1.78) |

a–dGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S8.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and log10-transformed somatic cell count (LogSCC) on milking liner slip in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.40 |  |
| FRP | 0.2 (0.1) |  | 1.07 (0.91–1.26) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.003 |  |
| Period 2 (FRP) | 0.2 (0.1)a |  | 1.55 (1.08−2.22) |
| Period 4 (FRP) | Referentab |  | 1.27 (0.87−1.86) |
| Period 1 (CON) | 0.4 (0.1)a |  | 1.51 (1.05−2.16) |
| Period 3 (CON) | 0.4 (0.1)a |  | 1.50 (1.04−2.15) |
| Period 5 (CON) | Referentb |  | − |
| Lactation number |  | 0.002 |  |
| 1st | –0.3 (0.1)b |  | 0.71 (0.58–0.86) |
| 2nd | –0.2 (0.1)ab |  | 0.83 (0.69–1.00) |
| ≥ 3rd | Referenta |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | −0.4 (0.1)c |  | 0.70 (0.56–0.87) |
| 101–200 DIM | 0.3 (0.1)a |  | 1.30 (1.09–1.55) |
| > 200 DIM | Referentb |  | – |
| Milking session |  | 0.02 |  |
| Session 1 | 0.2 (0.1)a |  | 1.24 (1.03–1.49) |
| Session 2 | −0.1 (0.1)b |  | 0.97 (0.80–1.18) |
| Session 3 | Referentab |  | – |
| LogSCC | 0.7 (0.1) | <0.0001 | 1.99 (1.76–2.26) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S9.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and log10-transformed somatic cell count (LogSCC) on milking unit kick-off in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.75 |  |
| FRP | 0.2 (0.3) |  | 1.05 (0.76–1.46) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.0002 |  |
| Period 2 (FRP) | 0.7 (0.3)a |  | 2.55 (1.23−5.29) |
| Period 4 (FRP) | Referentbc |  | 1.27 (0.56−2.89) |
| Period 1 (CON) | 1.0 (0.3)a |  | 2.60 (1.25−5.41) |
| Period 3 (CON) | 0.6 (0.3)ac |  | 1.91 (0.89−4.08) |
| Period 5 (CON) | Referentbc |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | 1.8 (0.4)a |  | 5.99 (2.72–13.20) |
| 2nd | 0.3 (0.5)b |  | 1.32 (0.51–3.40) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | 0.01 |  |
| ≤ 100 DIM | 1.0 (0.4)a |  | 2.71 (1.27–5.80) |
| 101–200 DIM | −0.2 (0.4)b |  | 0.85 (0.37–1.94) |
| > 200 DIM | Referentb |  | – |
| Milking session |  | 0.97 |  |
| Session 1 | −0.03 (0.2) |  | 0.98 (0.67–1.42) |
| Session 2 | 0.02 (0.2) |  | 1.02 (0.70–1.48) |
| Session 3 | Referent |  | – |
| LogSCC | 0.6 (0.3) | 0.04 | 1.80 (1.02–3.21) |

a–bGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S10.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, stage of lactation, and milking session on milking unit reattachment in Trial I

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.84 |  |
| FRP | −0.06 (0.1) |  | 1.02 (0.85–1.23) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.19 |  |
| Period 2 (FRP) | 0.3 (0.1) |  | 1.24 (0.83−1.84) |
| Period 4 (FRP) | Referent |  | 0.95 (0.62−1.45) |
| Period 1 (CON) | 0.2 (0.1) |  | 1.17 (0.79−1.74) |
| Period 3 (CON) | 0.02 (0.1) |  | 1.02 (0.68−1.54) |
| Period 5 (CON) | Referent |  | − |
| Stage of lactation |  | 0.0003 |  |
| ≤ 100 DIM | 0.1 (0.1)a |  | 1.11 (0.89–1.37) |
| 101–200 DIM | −0.4 (0.1)b |  | 0.69 (0.55–0.86) |
| > 200 DIM | Referenta |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −1.6 (0.1)a |  | 0.29 (0.22–0.37) |
| Session 2 | −0.8 (0.1)b |  | 0.47 (0.38–0.58) |
| Session 3 | Referentc |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S11.** Multivariable generalized linear mixed model showing the effect of treatment and period within the treatment on teat-end hyperkeratosis in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.87 |  |
| FRP | −0.06 (0.3) |  | 1.05 (0.38−2.87) |
| CON | Referent |  | − |
| Period (Treatment) |  | 0.86 |  |
| Period 2 (FRP) | 0.01(0.3) |  | 0.96 (0.10−9.37) |
| Period 4 (FRP) | Referent |  | 0.95 (0.09−10.51) |
| Period 1 (CON) | −0.2 (0.3) |  | 0.83 (0.09−8.01) |
| Period 3 (CON) | Referent |  | − |

a–bGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc test.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S12.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and the interaction between treatment and lactation number on milk yield (kg/session) in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.07 |  |
| FRP | ­­­−0.8 (0.2) |  | − |
| CON | Referent |  | − |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | 1.0 (0.1)a |  | 14.4 (13.9−14.9) |
| Period 4 (FRP) | Referentc |  | 13.4 (13.0−13.9) |
| Period 1 (CON) | 0.4 (0.1)a |  | 14.3 (13.8−14.8) |
| Period 3 (CON) | Referentb |  | 13.9 (13.4−14.4) |
| Lactation number |  | <0.0001 |  |
| 1st | −3.4 (0.5)b |  | − |
| 2nd | −0.3 (0.6)a |  | − |
| ≥ 3rd | Referenta |  | − |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 2.9 (0.5)a |  | 15.2 (14.4−16.0) |
| 101–200 DIM | 2.3 (0.5)a |  | 14.6 (13.7−15.5) |
| > 200 DIM | Referentb |  | 12.3 (11.6−12.9) |
| Milking session |  | <0.0001 |  |
| Session 1 | 3.2 (0.1)a |  | 16.7 (16.3–17.2) |
| Session 2 | −1.6 (0.1)c |  | 11.9 (11.4–12.3) |
| Session 3 | Referentb |  | 13.5 (13.0–13.9) |
| Treatment × Lactation number |  | 0.008 |  |
| FRP × 1st | 0.7 (0.2)c |  | 12.2 (11.5−12.8) |
| CON × 1st | Referentc |  | 12.0 (11.3−12.7) |
| FRP × 2nd | 0.2 (0.3)ab |  | 14.8 (13.9−15.8) |
| CON × 2nd | Referentab |  | 15.1 (14.1−16.0) |
| FRP × 3rd | Referentb |  | 14.8 (14.1−15.5) |
| CON × 3rd | Referenta |  | 15.3 (14.6−16.0) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S13.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session and log10-transformed somatic cell count (logSCC) on machine-on time (s) in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.43 |  |
| FRP | −0.01 (0.01) |  | 266 (258–274) |
| CON | Referent |  | 267 (259–275) |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | 0.02 (0.01)a |  | 273 (265−282) |
| Period 4 (FRP) | Referentc |  | 259 (251−268) |
| Period 1 (CON) | 0.01 (0.01)ab |  | 269 (261−278) |
| Period 3 (CON) | Referentb |  | 265 (257−273) |
| Lactation number |  | <0.0001 |  |
| 1st | −0.09 (0.01)b |  | 237 (226–247) |
| 2nd | ­­−0.02 (0.02)a |  | 276 (260–293) |
| ≥ 3rd | Referenta |  | 291 (278–304) |
| Stage of lactation |  | 0.04 |  |
| ≤ 100 DIM | 0.04 (0.01)a |  | 279 (264–295) |
| 101–200 DIM | 0.02 (0.01)ab |  | 267 (253–281) |
| > 200 DIM | Referentb |  | 255 (245–266) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.06 (0.01)a |  | 296 (287–305) |
| Session 2 | −0.02 (0.01)c |  | 247 (240–255) |
| Session 3 | Referentb |  | 260 (252–268) |
| LogSCC | −0.04 (0.01) | 0.001 | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S14.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, stage of lactation, milking session, and log10-transformed somatic cell count (logSCC) on 2-minute milk yield (kg) in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| FRP | −0.2 (0.1) |  | 5.9 (5.6–6.2) |
| CON | Referent |  | 6.1 (5.8–6.4) |
| Period (Treatment) |  | 0.003 |  |
| Period 2 (FRP) | 0.1 (0.1)bc |  | 5.9 (5.7−6.2) |
| Period 4 (FRP) | Referentc |  | 5.8 (5.5−6.1) |
| Period 1 (CON) | 0.2 (0.1)a |  | 6.2 (5.9−6.5) |
| Period 3 (CON) | Referentb |  | 6.0 (5.7−6.3) |
| Stage of lactation |  | 0.009 |  |
| ≤ 100 DIM | 0.9 (0.3)a |  | 6.3 (5.8–6.9) |
| 101–200 DIM | 0.8 (0.3)a |  | 6.2 (5.7–6.8) |
| > 200 DIM | Referentb |  | 5.4 (5.0–5.8) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.3 (0.1)a |  | 6.5 (6.2–6.8) |
| Session 2 | −0.8 (0.1)c |  | 5.4 (5.1–5.6) |
| Session 3 | Referentb |  | 6.2 (5.9–6.5) |
| LogSCC | 0.7 (0.2) | 0.006 | − |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S15.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, and milking session on peak milk flow rate (kg/min) in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.01 |  |
| FRP | 0.08 (0.03) |  | 5.1 (4.9–5.3) |
| CON | Referent |  | 5.0 (4.9–5.2) |
| Period (Treatment) |  | 0.7 |  |
| Period 2 (FRP) | −0.02 (0.03) |  | 5.1 (4.9−5.3) |
| Period 4 (FRP) | Referent |  | 5.1 (4.9−5.3) |
| Period 1 (CON) | 0.01 (0.03) |  | 5.1 (4.9−5.2) |
| Period 3 (CON) | Referent |  | 5.0 (4.9−5.2) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.1 (0.02)a |  | 5.2 (5.0–5.4) |
| Session 2 | −0.1 (0.02)c |  | 5.0 (4.8–5.1) |
| Session 3 | Referentb |  | 5.1 (4.9–5.2) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S16.** Multivariable general linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and log10-transformed somatic cell count (logSCC) on duration of low milk flow rate (s) in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| FRP | 0.1 (0.01) |  | 23 (21−24) |
| CON | Referent |  | 17 (16−18) |
| Period (Treatment) |  | 0.009 |  |
| Period 2 (FRP) | −0.02 (0.01)a |  | 22 (21−24) |
| Period 4 (FRP) | Referenta |  | 23 (22−25) |
| Period 1 (CON) | −0.02 (0.01)b |  | 17 (16−18) |
| Period 3 (CON) | Referentb |  | 18 (16−19) |
| Lactation number |  | 0.07 |  |
| 1st | 0.07 (0.03)a |  | 21 (19−23) |
| 2nd | 0.06 (0.03)ab |  | 21 (18−23) |
| ≥ 3rd | Referentb |  | 18 (16−20) |
| Stage of lactation |  | 0.0003 |  |
| ≤ 100 DIM | −0.1 (0.03)b |  | 18 (16−20) |
| 101–200 DIM | −0.1 (0.03)b |  | 18 (16−21) |
| > 200 DIM | Referenta |  | 23 (22−26) |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.1 (0.01)c |  | 16 (15−17) |
| Session 2 | 0.1 (0.01)a |  | 24 (23−26) |
| Session 3 | Referentb |  | 20 (19−22) |
| LogSCC | −0.05 (0.02) | 0.02 | − |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S17.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and the interaction between treatment and lactation number on bimodality in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | <0.0001 |  |
| FRP | −0.5 (0.1) |  | – |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.006 |  |
| Period 2 (FRP) | −0.1(0.04)a |  | 0.57 (0.51−0.64) |
| Period 4 (FRP) | Referenta |  | 0.61 (0.54−0.68) |
| Period 1 (CON) | −0.1 (0.04)b |  | 0.89 (0.79−0.99) |
| Period 3 (CON) | Referentc |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | 0.04 (0.05)b |  | – |
| 2nd | 0.6 (0.05)a |  | – |
| ≥ 3rd | Referentc |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | −1.4 (0.1)c |  | 0.26 (0.24–0.28) |
| 101–200 DIM | −0.7 (0.1)b |  | 0.51 (0.48–0.55) |
| > 200 DIM | Referenta |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.1 (0.04)c |  | 0.88 (0.82–0.96) |
| Session 2 | 1.1 (0.04)a |  | 2.97 (2.76–3.19) |
| Session 3 | Referentb |  | – |
| Treatment × Lactation number |  | 0.01 |  |
| FRP × 1st | 0.2 (0.1)c |  | 0.71 (0.61–0.82) |
| CON × 1st | Referentb |  | – |
| FRP × 2nd | −0.1 (0.1)b |  | 0.57 (0.48–0.68) |
| CON × 2nd | Referenta |  | – |
| FRP × 3rd | Referentd |  | 0.60 (0.52–0.70) |
| CON × 3rd | Referentb |  | – |

a–dGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S18.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, session, and interaction between treatment and lactation number on milking liner slip in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.01 |  |
| FRP | 0.1 (0.1) |  | – |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.005 |  |
| Period 2 (FRP) | −0.2(0.1)ab |  | 0.96 (0.81−1.15) |
| Period 4 (FRP) | Referenta |  | 1.13 (0.95−1.36) |
| Period 1 (CON) | −0.2 (0.1)b |  | 0.85 (0.70−1.02) |
| Period 3 (CON) | Referentab |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | −0.5 (0.1)b |  | – |
| 2nd | 0.1 (0.1)a |  | – |
| ≥ 3rd | Referenta |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 0.5 (0.1)a |  | 1.64 (1.46–1.84) |
| 101–200 DIM | 0.1 (0.1)b |  | 1.10 (0.97–1.26) |
| > 200 DIM | Referentb |  | – |
| Milking session |  | 0.004 |  |
| Session 1 | 0.1 (0.1)a |  | 1.09 (0.97–1.23) |
| Session 2 | −0.1 (0.1)b |  | 0.89 (0.79–1.01) |
| Session 3 | Referentab |  | – |
| Treatment × Lactation number |  | 0.03 |  |
| FRP × 1st | 0.2 (0.1)a |  | 1.35 (1.04–1.75) |
| CON × 1st | Referentb |  | – |
| FRP × 2nd | −0.2 (0.1)c |  | 0.94 (0.71–1.25) |
| CON × 2nd | Referentc |  | – |
| FRP × 3rd | Referentc |  | 1.16 (0.93–1.44) |
| CON × 3rd | Referentc |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S19.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, milking session, and log10-transformed somatic cell count (LogSCC) on milking unit kick-off in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.02 |  |
| FRP | 0.4 (0.1) |  | 1.28 (1.04–1.57) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.01 |  |
| Period 2 (FRP) | −0.4(0.1)a |  | 0.99 (0.67−1.45) |
| Period 4 (FRP) | Referentb |  | 1.51 (1.06−2.17) |
| Period 1 (CON) | −0.1 (0.1)a |  | 0.92 (0.61−1.38) |
| Period 3 (CON) | Referenta |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | 2.7 (0.2)a |  | 15.58 (10.46–23.22) |
| 2nd | 0.5 (0.3)b |  | 1.70 (0.96–3.03) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 2.3 (0.2)a |  | 9.73 (6.96–13.61) |
| 101–200 DIM | 0.6 (0.2)b |  | 1.86 (1.27–2.74) |
| > 200 DIM | Referentc |  | – |
| Milking session |  | 0.2 |  |
| Session 1 | −0.2 (0.1) |  | 0.85 (0.69–1.04) |
| Session 2 | −0.2 (0.1) |  | 0.85 (0.69–1.04) |
| Session 3 | Referent |  | – |
| LogSCC | 0.5 (0.1) | 0.0002 | 1.59 (1.25–2.01) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S20.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, and milking session on milking unit reattachment in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.01 |  |
| FRP | 0.3 (0.1) |  | 1.17 (1.04–1.32) |
| CON | Referent |  | – |
| Period (Treatment) |  | 0.07 |  |
| Period 2 (FRP) | −0.1(0.1)ab |  | 1.16 (0.93−1.44) |
| Period 4 (FRP) | Referentb |  | 1.34 (1.08−1.67) |
| Period 1 (CON) | 0.1 (0.1)ab |  | 1.14 (0.91−1.44) |
| Period 3 (CON) | Referenta |  | − |
| Lactation number |  | <0.0001 |  |
| 1st | 0.4 (0.1)a |  | 1.51 (1.32–1.72) |
| 2nd | −0.2 (0.1)b |  | 0.82 (0.68–0.98) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 0.2 (0.1)a |  | 1.25 (1.09–1.43) |
| 101–200 DIM | −0.4 (0.1)c |  | 0.69 (0.59–0.82) |
| > 200 DIM | Referentb |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.8 (0.1)b |  | 0.44 (0.37–0.51) |
| Session 2 | −0.1 (0.1)a |  | 0.90 (0.79–1.03) |
| Session 3 | Referenta |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S21.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, lactation number, stage of lactation, and log10-transformed somatic cell count (LogSCC) on stepping activity of hindlegs during milking in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| FRP | 0.2 (0.05) |  | 5.8 (5.2–6.4) |
| CON | Referent |  | 4.9 (4.4–5.4) |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | −0.2 (0.1)b |  | 5.1 (4.6−5.8) |
| Period 4 (FRP) | Referenta |  | 6.5 (5.8−7.3) |
| Period 1 (CON) | −0.2 (0.05)c |  | 4.5 (4.0−5.0) |
| Period 3 (CON) | Referentb |  | 5.3 (4.7−6.0) |
| Lactation number |  | <0.0001 |  |
| 1st | −0.7 (0.1)c |  | 3.8 (3.3–4.4) |
| 2nd | −0.4 (0.1)b |  | 5.1 (4.2–6.3) |
| ≥ 3rd | Referenta |  | 7.6 (6.6–8.8) |
| Stage of lactation |  | 0.001 |  |
| ≤ 100 DIM | −0.4 (0.1)b |  | 4.1 (3.4–4.9) |
| 101–200 DIM | 0.05 (0.1)a |  | 6.2 (5.2–7.3) |
| > 200 DIM | Referenta |  | 5.9 (5.2–6.8) |
| LogSCC | −0.2 (0.1) | 0.008 | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Log estimates of regression.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).

**Supplemental Table S22.** Multivariable generalized linear mixed model showing the effect of treatment, period within the treatment, and lactation number on kicking activity of hindleg during milking in Trial II

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.001 |  |
| FRP | 0.4 (0.1) |  | 1.1 (1.0–1.4) |
| CON | Referent |  | 0.9 (0.7–1.1) |
| Period (Treatment) |  | <0.0001 |  |
| Period 2 (FRP) | −0.6 (0.1)bc |  | 0.9 (0.7−1.1) |
| Period 4 (FRP) | Referenta |  | 1.5 (1.2−1.9) |
| Period 1 (CON) | −0.3 (0.1)c |  | 0.8 (0.6−0.9) |
| Period 3 (CON) | Referentb |  | 1.1 (0.9−1.3) |
| Lactation number |  | 0.002 |  |
| 1st | 0.6 (0.2)a |  | 1.2 (1.0–1.6) |
| 2nd | 0.6 (0.2)a |  | 1.3 (0.9–1.8) |
| ≥ 3rd | Referentb |  | 0.7 (0.5–0.9) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Log estimates of regression.

3Treatment consisted of 2 different premilking stimulation regimens: flow-responsive pulsation (FRP) and conventional (CON).