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A single prolonged milking interval of 24 h compromises the well-being and health of dairy Holstein cows

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ABSTRACT

Cows are often shown at dairy shows with overfilled udders to achieve a better show placing. However, it is unclear to what degree "over-bagging" affects the health and well-being of show cows. The goal of this study was to assess the effect of a single prolonged milking interval (PMI) of 24 h on the measurable signs of health and well-being in dairy cows in early and mid-lactation and to assess the effect of a nonsteroidal anti-inflammatory drug (NSAID) on well-being during a PMI. Fifteen Holstein cows were studied in early lactation (89.5 ± 2.7 d in milk) and were given an NSAID or physiological saline in a crossover design. Ten cows were studied again in mid-lactation (151.6 ± 4.0 d in milk). Data on clinical signs of cows' health, behavior, and well-being were collected at 1 or 2 h intervals before and during a PMI of 24 h. Data from the last 6 h of a 12 h milking interval were compared with the last 6 h of the PMI. Compared with that of a cow in the last 6 h of a 12-h milking interval, the behavior of cows in early lactation (saline group) changed during the last 6 h of the PMI: we observed decreased eating time (22.4 vs. 16.2 min/h), increased ruminating time (13.3 vs. 25.0 min/h), and increased hind limb abduction while walking (score 41.7 vs. 62.6) and standing (31.2 vs. 38.9 cm). Udder firmness was increased (2.9 vs. 4.5 kg) during this period and more weight was placed on the hind limbs (46.4 vs. 47.0%). We also found pathological signs at the end of the PMI: all cows showed milk leaking, and 10 of 15 cows developed edema in the subcutaneous udder tissue. Somatic cell count was significantly increased from 12 h to 72 h after the PMI. Administration of an NSAID had no influence on measured variables, except that the occurrence of edema was not significantly increased during PMI in the flunixin group (10 of 15 and 6 of 15 cows for the saline and flunixin groups, respectively). In the cows in mid-lactation, different variables were not significantly changed in the PMI compared with baseline values (e.g., eating and ruminating time, occurrence of edema, and abduction). We conclude that the cows' health and well-being were compromised by a single PMI of 24 h, because their behavior changed and pathological signs were recorded. Administration of an NSAID had a slight effect on cows' well-being during a PMI. The stage of lactation had more effect on the cows' health and well-being, because fewer variables were changed in mid-lactation.
Key words: prolonged milking interval, animal well-being, over-bagging, dairy cow show

INTRODUCTION

Cows at national and international dairy cow shows are often presented with extremely filled udders in order to attain a better show placing. This "over-bagging" of the udder might be painful, and therefore be an animal welfare issue (Gleeson et al., 2007; Kohler and Steiner, 2013). Freedom from discomfort and from pain are 2 of the 5 freedoms stated in the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE, 2015). Over-bagging and the unnecessary suffering of show cattle is against the showing regulations of many show organizations around the world, including Switzerland (ASR, 2011), the United Kingdom (HUK, 2011), and the United States (WDE, 2015). However, guidelines are formulated vaguely and lack objective control methods to assess animal welfare and over-bagging. Over-bagging is said to be one of the most severe problems still to be solved in show ethics (Geiger, 2015). Currently, over-bagging is either not enforced as a violation of dairy shows' code of ethics, or it is enforced by subjective assessment of cows' behavior by a show veterinarian. In a preliminary study (Kohler and Steiner, 2013), assessment of well-being by veterinarians and show judges, using visual analog scales to describe well-being and udder fill, proved to be

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very subjective and not a feasible tool for monitoring an animal's well-being. It is presumed that nonsteroidal anti-inflammatory drugs (**NSAID**) are used to cover the signs of impaired well-being in over-bagged show cows, and this practice complicates the subjective detection of over-bagged cows. Nonsteroidal anti-inflammatory drugs are usually administered at the last milking before the cow show, because it is not allowed to use these substances for this indication at the show site (personal communication, A. Wyss, Federal Food Safety and Veterinary Office, Berne, Switzerland).

A single prolonged milking interval (**PMI**) of 24 h may cause an increase in SCC by inducing an inflammatory reaction and increasing the permeability of the udder (Lakic et al., 2009, 2011). The blood–milk barrier switches to a leaky state at approximately 18 h after the last milking (Stelwagen et al., 1997). Additionally, a PMI can lead to edema in the subcutaneous udder tissue (Waller et al., 2007). Cows show higher locomotion scores and more cows show milk leakage in once-a-day milking systems compared with twice-a-day milking systems; furthermore, udder firmness is increased in these cows (Tucker et al., 2007). Gait score and weight distribution are also affected by milking (Chapinal et al., 2009). Gait score is increased and more weight is placed on the hind limbs 1 h before milking, compared with immediately after milking. As well, abrupt drying off causes increased extramammary pressure, and—in high-yielding cows—even increased fecal glucocorticoid concentration (Bertulat et al., 2013), indicating that over-bagging is stressful.

The objectives of the current study were to investigate the effect of a sudden change to a PMI of 24 h in early and mid-lactation, and to investigate the effect of NSAID administered before the PMI on several variables of animal health and well-being. We hypothesized that during the last 6 h of the PMI, behavior would be different, and that udder firmness, udder surface temperature, edema in the subcutaneous udder tissue and milk leakage would be increased compared with the control period. Additionally, we expected an increase in SCC after a single PMI of 24 h.

MATERIALS AND METHODS

Animals, Housing, and Milking

The study was conducted at an agricultural research station (Agroscope Liebefeld-Posieux, Posieux, Switzerland) from May to December 2014. The 15 cows (10 Holstein Friesian and 5 Red Holstein) studied in early lactation averaged 89.5 ± 2.7 DIM (mean ± SD), and parity was 2.8 ± 1.0 (age 4.7 ± 1.2 yr). The cows yielded 31.0 ± 7.0 kg [interquartile range (**IQR**) 25.3–34.9 kg] milk/d (305-d milk yield 7,364 ± 1,040 kg; IQR 6,440–8,130 kg) and had a BW of 681.5 ± 68.8 kg (IQR 628.5–712.0 kg). The 10 cows studied in mid-lactation averaged 151.6 ± 4.0 DIM, and parity was 2.6 ± 0.9 (age 4.6 ± 1.3 yr). They yielded 28.4 ± 4.3 kg (IQR 26.0–32.1 kg) milk/d (305 d milk yield 7,310 ± 1,057 kg; IQR 6,512–7,829 kg) and had a BW of 684.4 ± 79.6 kg (IQR 612.8–713.0 kg). During the experiments, cows were individually housed in a tie-stall barn and had free access to water, offered in individual drinking bowls. Cows were fed hay and corn silage (summer feeding) or TMR (winter feeding) and received additional concentrate and mineral feed according to their current milk yield. The experimental protocol was approved by the Ethics Committee for Animal Experimentation of the Canton of Fribourg, Switzerland (#2014_08_FR).

During the study, cows were always milked by the first author (PK) using a bucket milking system (SAC, Kolding, Denmark) at 0500 and 1700 h. The milking cluster was attached after a pre-stimulation time of 2.5 min. During pre-stimulation, the udder was cleaned, the California Mastitis Test was performed, and separate aseptic milk samples were taken from each quarter. Post-milking stimulation started when milk flow decreased to <0.2 kg/min. After a short manual stimulation, the cluster was detached and the teats were dipped with Mammo-Derm (Multisforsa, Auw, Switzerland).

Experimental Design

The study consisted of 3 experiments. Experiment 1 and 2 were performed in early lactation (90 DIM; n = 15 cows) and experiment 3 in mid-lactation (150 DIM; n = 10 cows), as shown in Figure 1. After an adaptation period of 3 d with milking intervals of 12 h and habituation to the experimental procedure (cows underwent examinations 3 times per day), experiments generally consisted of 3 periods: a baseline period (**BLP**) of 24 h with milking intervals of 12 h; a PMI of 24 h; and a recovery period (**RP**) of 12 h. In the crossover design of experiments 1 and 2, cows were administered either flunixin meglumine (Fluniximin; Dr. E. Graeub AG, Berne, Switzerland; 2.2 mg/kg BW, i.v.) or an equivalent volume of sterile physiological saline solution i.v. at the beginning of the PMI. The interval between the end of experiment 1 and the beginning of experiment 2 was 7 d. The BLP was not repeated in experiment 2, and for statistical analyses, data from the BLP of experiment 1 were used for experiment 2. For experiment 3, 10 of the 15 study cows were randomly selected. The experimental design was similar to experiment 1, except that all cows were administered sterile physiological saline solution. Data collection during the various periods was performed according to Figure 1.

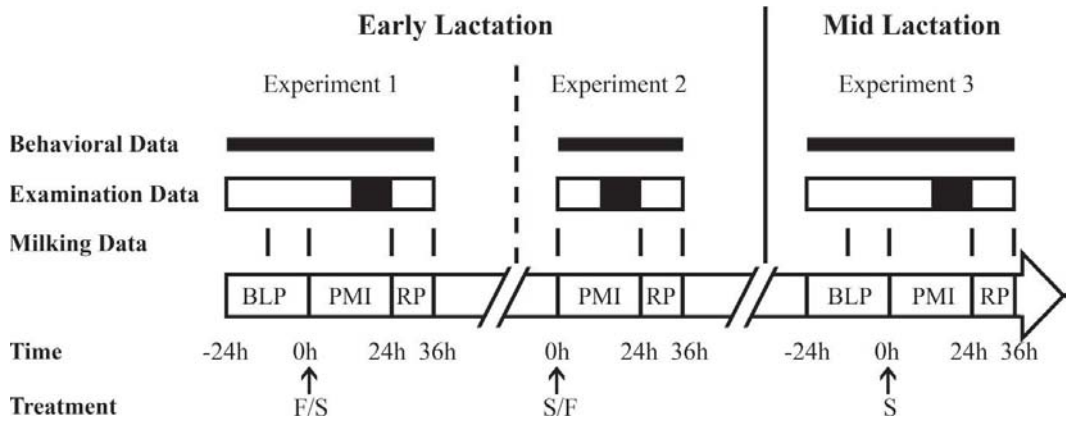

Figure 1. Schematic representation of the experimental design and the data collection schedule of experiments 1 to 3: baseline period (BLP), prolonged milking interval (PMI), and recovery period (RP). Behavioral data included locomotion and feeding behavior and were recorded continuously. Examination data included all periodically taken data (e.g., gait score, weight distribution, udder firmness, and other variables) and were collected every 2 h (open bar) or every hour (black bar) during hours 17 to 24 of the PMI. Milking data included milk flow curve, electrical conductivity, and SCC and were collected at each milking during the experiment (|). Treatments are marked with an arrow [↑; F/S = cow received either flunixin (F) or sterile physiologic saline solution (S) in a crossover design].

Inclusion Criteria

Cows were examined clinically according to Dirksen and Stöber (2006) 3 d before entering the experiments. All organ systems were examined. Only healthy cows were used in the experiments, including no increase in rectal temperature (<39.5°C), no signs of gastrointestinal disorders or infections of the respiratory tract, no lameness [gait score ≤2/5 according to Flower and Weary (2006), as assessed by the first author, while cows were walking along a passageway of 12.5 × 13 m on concrete floor], no increase in SCC [<150,000 cells/mL measured with DeLaval cell counter DCC (DeLaval, Tumba, Sweden); Bates et al., 2016].

Parameters and Data Collection

Data were collected in different intervals. Data for monitoring the locomotion and feeding behavior were collected continuously throughout the experiments. Examination data (heart rate, respiratory rate, salivary cortisol, gait score, udder firmness, superficial udder temperature, udder width, and edema of the subcutaneous tissue) were collected every 2 h or every 1 h during h 17 to 24 of the PMI. Milking data were collected at every milking during the experiment (Figure 1).

Locomotion and Feeding Behavior

Three days before the experiments, cows were equipped with a pedometer and a noseband sensor from the RumiWatch system (ITIN & HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) to assess locomotion and feeding behavior, as validated by Alsaaod et al. (2015) and Ruuska et al. (2016). The following variables of locomotion were calculated: lying time (min/h) and limb events (movements of the legs or <3 strides, no./h). The following variables of feeding behavior were calculated: eating time (min/h), eating chews (no./h), ruminating time (min/h), ruminating chews (no./h), boluses (no./h), chews per bolus (no.), and activity change (changing between activities of eating, ruminating, and no activity). When examination data were collected in a 2-h interval (Figure 1), data from the hour in which the cow was examined (first hour) were used for the analysis and data from the second hour were discarded. This was done to exclude hours in which the cows were not examined and to allow for comparison between the 1 and 2 h examination intervals. Walking behavior [stride duration (ms) and stride distance (cm)] were calculated using only data collected during walking from the stall to the 4-scale weighing platform (approximately 25 m).

Examination Data

Collection of all examination data took approximately 20 min and was performed every 1 or 2 h (Figure 1). The different variables were assessed in the order they are described. All data except gait scoring and weight distribution were collected in the tiestall barn. Gait score was assessed as the cows were walking along a passageway outside of the barn. The scale for the assessment of weight distribution was placed at the end of this passageway.

Heart Rate, Respiratory Rate, Temperature, and Milk Leaking. Heart rate was assessed by cardiac auscultation, respiratory rate by observation of thoracic

movements and rectal body temperature was measured with a rectal thermometer. The ambient temperature was assessed by a thermometer to control for variation during measurements of superficial udder temperature. Cows were visually checked for signs of milk leaking without touching the udder.

Salivary Cortisol. Saliva samples were collected using synthetic swabs (Salivette; Sarstedt, Nümbrecht, Germany) by pushing the swabs into the cow's mouth and letting the cow chew on the swab for approximately 10 s. Samples were immediately stored on ice until they were frozen at −20°C. After thawing, samples were centrifuged for 6 min at 3,000 × *g*, and salivary cortisol concentration was measured using a direct enzyme immunoassay kit (Salimetrics LCC, Carlsbad, CA) in the laboratory (Institute of Veterinary Physiology, Vetsuisse-Faculty, University of Bern, Switzerland). This method has been described previously and was adapted for use in cows (Schwinn et al., 2016).

Gait Score. To assess gait, cows were videotaped (Canon HF100; Canon, Tokyo, Japan) walking along the passageway. Gait was analyzed by 3 trained independent observers (PK, MA, AS); they scored the videos in random order using a numeric rating scale (1–5, **NRS**) from Flower and Weary (2006) to assess lameness and a visual analog scale (0–100; **VAS**) to subjectively assess the degree of abduction of the hind limbs. In the VAS, 0 equaled the minimal imaginable abduction, 50 the abduction expected to occur before milking after a 12 h milking interval, and 100 the maximal imaginable abduction. Examiners were blinded to the interval since the last milking and treatment.

Weight Distribution Among Limbs. Weight distribution was measured while cows were standing on a 4-scale weighing platform (1.94 × 1.06 m; ITIN & HOCH GmbH, Fütterungstechnik) situated at the end of the passageway. The platform consisted of 4 recording units (0.78 × 0.55 m), as described by Nechanitzky et al. (2016). The weight of each leg delivered to the respective unit was recorded for 5 min with a frequency of 10 Hz. The following variables were calculated for each 5 min measurement: difference between front and hind limbs, percentage of BW placed on the hind limbs, and the standard deviation of the weight of each limb.

Udder Firmness. Udder firmness was determined using a digital dynamometer (Agrosta DFT 15; Agro Technologies, Forges-les-Eaux, France) following the guidelines described by Bertulat et al. (2012): the centers of both hind quarters were marked using Raidex animal marker spray (Raidex GmbH, Dettingen, Germany). Measurements were performed by pressing a round tip (16 mm diameter) at a right angle to the udder surface and measuring the maximal force of penetration (kg). Penetration depth was set to 2 cm by a plate mounted on the dynamometer (10.3 × 7.2 cm). The firmness of each hindquarter was determined 5 times in a row. For all measurements, both hind limbs had to be in a parallel position and not be moved. The coefficient of variation among measurements must not have exceeded 10%. Otherwise, the complete set of measurements was repeated. Because we did not find any differences between hindquarters, we used the mean of the 10 measurements of both quarters for further analysis.

Superficial Udder Temperature. Infrared images were taken using a thermal imager (Fluke Ti200; Fluke IR-Fusion Technology, Everett, WA; uncooled micro-bolometer, 200 ×150 pixel, thermal sensitivity <0.075°C at 30°C) of the caudal aspect of the udder while holding the tail to the side. Images were taken at a distance of 1.5 m, as measured by a laser range finder (LDM 50 T; Toolcraft, Hirschau, Germany). The emissivity of the thermal imager was set at 0.95. Udder surface temperature and skin surface temperature (of a 10 × 10 cm clipped area 20 cm proximal to the tuber calcanei) were determined using Smart View 3.7 software (Fluke IR-Fusion Technology, Everett, WA). The maximal values of the areas of interest were used for the analysis as suggested by Metzner et al. (2014) for the detection of mastitis and Alsaaod et al. (2014) for the detection of digital dermatitis. Maximal skin surface temperature was used to normalize for ambient temperature differences.

Udder Width and Limb Distance. Udder width was defined as the maximal horizontal width of the udder and limb distance as the maximal horizontal distance between the medial surfaces of the metacarpi. With the thermal imager, a digital photograph was taken of the udder and both hind limbs to determine udder width and limb distance. Images had a resolution of 2,560 × 1920 (5 megapixels). Both hind limbs had to be parallel for the picture; otherwise, the cow was manipulated until limbs were in a parallel position. A 5-cm scale was mounted on the pedometers to allow correct determination of the absolute values of udder width and limb distance, using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA).

Sonographic Appearance of the Subcutaneous Udder Tissue. Ultrasound images were recorded using a portable ultrasound scanner (Imago; Echo Control Medical, Angoulême, France) with a 7.5-MHz linear rectal probe (LB760P) to detect edema in the subcutaneous tissue. Maximal depth and focus were set at 4 and 2.5 cm, respectively. The lateral aspects of the forequarters and the mid-height median crease around the suspensory ligament were scanned for the presence of subcutaneous edema, because these regions are where edema due to over-bagging is found most frequently

(Waller et al., 2007). An oil gel solution (Johnson & Johnson, New Brunswick, NJ) was used as coupling agent, because it is routinely used as a gloss agent for udders at dairy cow shows. One image of each of the 3 regions described above was stored and analyzed using Synedra View Personal version 3.4 (Synedra Information Technologies GmbH, Innsbruck, Austria) to determinate the occurrence and measure the depth of edema. The maximal depth of edemas in the 3 regions (mm) were summed for each cow separately and used as 1 value per cow for further analysis. The occurrence of edema (yes/no) was also analyzed.

Milking Data

Milk Flow Curves and Electrical Conductivity. Milk flow curves and electrical conductivity were recorded continuously using a Lactocorder (WMB AG, Balgach, Switzerland) at each milking. To prevent bias, the milking procedure was standardized as described earlier, and data were excluded if the cluster was knocked off during milking (n = 9 milkings). Data were exported and processed using Lactopro software (WMB AG), and the following variables were used for further analysis: milk yield (kg); duration of incline, plateau and decline phases (min); time of peak milk flow (min); peak milk flow (kg/min); electrical conductivity during incline phase (mS/cm); and maximal electrical conductivity of the total milking (mS/cm).

SCC. A milk sample of the total milking was taken at every milking during the experiment and at the 4 evening milkings thereafter (+24, +48, +72, and +96 h); milk samples were not collected from cow 1 (experiments 1 and 2) and cows 2 and 3 (experiment 1). We measured SCC using a DeLaval Cell counter DCC (DeLaval), as described by Sarikaya and Bruckmaier (2006) and Kawai et al. (2013). Data from 1 cow were excluded from the analysis of SCC due to an infection with a major pathogen (*Streptococcus uberis*), because major pathogens can cause severe increases in SCC. Cows with minor pathogen infections were not excluded from the analysis.

Bacteriological Culture. Single-quarter milk samples were taken aseptically at each milking during the experiment; milk samples were not collected from cow 1 (experiment 1 and 2) and cows 2 and 3 (experiment 1). These milk samples were frozen at −20°C immediately after sampling. After thawing, the quarter milk samples from each milking were pooled to obtain 1 sample per cow per milking. It has been shown that using composite milk samples from 4 quarters is adequate for detecting IMI (Reyher and Dohoo, 2011). Aerobe bacteriological culture was performed according to standard procedures (Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Berne, Berne, Switzerland). Only samples taken directly before the PMI and 12 h after the PMI were analyzed. Samples before the PMI were used as controls and compared with the samples from 12 h after the PMI, because the peak of SCC was measured at 12 h after PMI. Bacteriological infections were classified as being caused by minor (e.g., *Corynebacterium bovis*, coagulase-negative staphylococci) or major (e.g., *Streptococcus uberis*) pathogens.

Statistical Analyses

Statistical analyses were carried out using STATA 14 (StataCorp LP, College Station, TX). For all variables collected, descriptive statistics were developed. All data were tested for normality using the Shapiro-Francia test. To add all observations of all parameters to the analysis, we tried to model our data in a general estimating equations model, but because the study contained repeated measurements over time, the different observations might not be independent, and possible correlations should be considered in the analysis. In our case, that was a panel model with an autoregressive (ar1) correlation structure. Due to the large number of predictors and the small sample size, it was not possible to estimate these correlations. Therefore, we tested variables against each other using common statistical tests as described below, knowing that some information might be lost using this procedure. Paired *t*-tests or Wilcoxon signed rank tests were used for normally and non-normally distributed variables, respectively. Frequency tables (occurrence of edema and milk leaking) were analyzed using Fisher's exact test with Cramer's V to measure association. For correlation between the VAS of abduction and udder width of hind limb distance, we used Spearman correlations ($\mathbf{r_s}$). The significance level was set at $P < 0.05$. All *P*-values were adjusted using the Bonferroni correction for multiple testing, separately for the different data sets (behavior and examination data, slope of examination data, and milking data). Post hoc power calculation was performed using G*Power version 3.1.9.2 (http://www.gpower.hhu.de/). The values for α and sample size were set at 0.05 and 15 cows, respectively. The actual values for differences of the means and standard deviation of the differences of the means were used to calculate the achieved power of the tests.

Grouping. For statistical analyses, the data from each experiment and each cow were assigned to 1 of the following 3 groups: early lactation administered saline (saline group; n = 15), early lactation administered flunixin meglumine (flunixin group; n = 15), or mid-

lactation administered saline (mid-lactation group; n = 10). Each group was analyzed on its own and compared with the other groups.

Comparison of BLP and PMI. For all variables of the behavioral and examination data, means of the last 6 h in the BLP (3 observations, 6–12 h after milking) and the last 6 h in the PMI (6 observations, 18 to 24 h after milking) were formed and compared against each other.

In the descriptive statistics for a few variables (weight distribution, udder firmness, VAS of abduction, udder width, and limb distance), a plateau at the end of the PMI was observed visually. To confirm this plateau, we compared the increase per hour (slope) of the respective variables during the last 6 h of the PMI (6 observations) with the corresponding 6 h before milking in the BLP (3 observations). For this comparison, local linear regressions for each cow and parameter during this period were formed, and the regression coefficients (slopes) were compared between the BLP and PMI.

Comparison of BLP and RP. The means of the first 12 h of the BLP (6 observations, 1–12 h after milking) were compared with the corresponding means of the RP (6 observations, 1–12 h after milking).

Milking Data. All data collected at the last milking of the BLP were compared with data from the milking at the end of the PMI. Because SCC was collected for 4 additional days after the PMI, an ANOVA with repeated measures was performed to detect changes in SCC, using Greenhouse-Geisser correction to interpret the significance level.

Comparison of Groups. All variables measured during the PMI of the saline group in early lactation were compared with the flunixin group in early lactation and the group in mid lactation, respectively.

RESULTS

Early-Lactation Saline Group

Behavioral Changes and Salivary Cortisol Concentration. Eating time decreased from 22.4 ± 4.8 min/h in the BLP to 16.2 ± 3.8 min/h in the PMI ($P < 0.001$). In contrast, ruminating time increased from 13.3 ± 6.0 to 25.0 ± 2.8 min/h ($P < 0.001$). Other variables of feeding behavior (eating chews, ruminating chews and ruminating boluses) supported these findings (Table1).

We found no differences in locomotion behavior (lying time and limb events) or walking attributes (stride duration and stride distance) between the BLP and PMI (Table 1). Likewise, we found no changes in salivary cortisol concentration between the BLP and PMI (data not shown).

Weight Distribution and Gait Score. The percentage of weight on the hind limbs increased from 46.4 ± 0.8% in the BLP to 47.0 ± 1.0% in the PMI ($P < 0.001$). The increase per hour (slope) of percentage of weight on hind limbs over the last 6 h before milking was higher in the BLP than in the PMI (0.10 ± 0.13 vs. −0.03 ± 0.07%/h; $P = 0.001$). The absolute difference between front and hind limbs changed similarly between the BLP and PMI (Tables 1 and 2). We found no differences in standard deviation of weight per limb (data not shown).

The VAS of abduction was 41.7 ± 15.6 in the BLP and increased to 62.6 ± 15.1 in the PMI ($P < 0.001$). The VAS increase per hour (slope) was higher in the BLP than in the PMI (4.1 ± 3.3 vs. 1.0 ± 1.8/h; $P = 0.006$). Udder width and hind limb distance showed a strong positive correlation with the VAS ($r_s = 0.632$ and $r_s = 0.626$, respectively; $P < 0.001$). The NRS revealed no differences between the BLP and PMI (Table 1).

Udder Firmness, Udder Surface Temperature, and Hind Limb Abduction. Udder firmness was 2.9 ± 0.7 kg in the BLP and increased to 4.5 ± 0.9 kg in the PMI ($P < 0.001$). The increase of udder firmness per hour (slope) was significantly higher in the BLP than in the PMI (0.23 ± 0.21 vs. 0.04 ± 0.21 kg/h; $P = 0.007$; Figure 2). For udder surface temperature, we found no differences between the BLP and PMI (data not shown).

Udder width and hind limb distance in BLP were 31.7 ± 3.9 and 31.2 ± 5.9 cm and increased in the PMI to 38.0 ± 4.4 and 38.9 ± 5.7 cm, respectively ($P < 0.001$). We found no differences in the increase per hour (slope) of udder width and limb distance between the BLP and PMI (Table 2).

Edema and Milk Leaking. Edema occurred in 0/15 cows in the BLP and in 10/15 cows in the PMI ($P < 0.001$; Cramer's V = 0.71). The average of cumulated depth of edema was 0.0 ± 0.0 mm in the BLP and increased to 1.2 ± 1.5 mm in the PMI ($P < 0.002$). The peak values of edema depth were 12.5 and 7.7 mm for cumulative depth and depth at a single location, respectively. Milk leaking occurred in 1/15 cow in the BLP and in 15/15 cows in the PMI ($P < 0.001$; Cramer's V = 0.96).

Milk Flow Curves, Electrical Conductivity, and SCC. Milk yield per milking was significantly lower in the BLP than in the PMI (14.4 ± 3.4 vs. 22.7 ± 6.4 kg; $P < 0.001$). However, milk yield per 24 h was higher in the BLP than in the PMI (30.7 ± 6.8 vs. 22.7 ± 6.4 kg; $P < 0.001$). Milk yield over 12 h in the BLP was also higher than over 12 h in the RP (16.1 ± 3.8 vs. 14.4 ± 3.3 kg; $P = 0.005$). The duration of the milk flow curve plateau phase of 2.8 ± 1.9 min in the BLP increased to 4.9 ± 3.4 min in the PMI ($P = 0.002$), but we found

Table 1. Behavioral and examination data from the early-lactation saline group (n = 15) for the baseline period and the prolonged milking interval

	Baseline period[1]			Prolonged milking interval[1]				
Variable	Mean ± SD	Median	Interquartile range	Mean ± SD	Median	Interquartile range	*P*-value[2]	Power
Eating time (min/h)	22.4 ± 4.8	21.5	20.2–25.7	16.2 ± 3.8	14.8	14.3–19.3	<0.001*	0.98
Eating chews (no./h)	1,355.4 ± 392.9	1,368.7	1,077.3–1,536.7	1,028.9 ± 376.0	898.0	819.4–1,205.8	0.013	0.81
Ruminating time (min/h)	13.3 ± 6.0	13.3	10.1–15.5	25.0 ± 2.8	25.5	22.8–27.0	<0.001*	1.00
Ruminating chews (no./h)	839.3 ± 436.5	827.3	591.0–987.8	1,583.8 ± 185.1	1,596.8	1,452.1–1,700.8	0.001*	1.00
Ruminating bolus (no./h)	14.6 ± 6.5	15.7	10.0–17.0	27.7 ± 2.7	27.2	25.6–29.8	<0.001*	1.00
Chews per ruminating bolus (no.)	53.5 ± 6.9	51.6	48.3–57.8	56.2 ± 5.9	56.3	53.1–59.2	0.046	0.53
Change of feeding activity[3] (no./h)	11.3 ± 4.1	11.7	8.7–14.2	8.8 ± 2.5	8.8	7.3–10.0	0.003	0.91
Lying time (min)	11.1 ± 5.5	10.8	7.2–14.3	8.5 ± 4.8	7.0	4.4–12.1	0.231	0.22
Limb events[4] (no./h)	87.4 ± 29.8	83.0	66.5–98.3	104.9 ± 32.0	104.3	91.0–117.8	0.010	0.66
Stride duration (ms)	1,857.8 ± 240.6	1,860.1	1,704.2–2,031.7	1,949.6 ± 223.6	1,920.6	1,856.6–2,045.3	0.061	0.55
Stride distance (cm)	153.5 ± 25.9	147.0	135.3–174.0	145.5 ± 26.7	143.1	126.4–158.7	0.081	0.40
Relative weight on hind limb (%)	46.3 ± 0.8	46.3	45.7–46.9	47.0 ± 1.0	46.9	46.5–47.8	<0.001*	1.00
Difference between front and hind limbs (kg)	24.9 ± 6.6	26.0	19.8–29.7	20.8 ± 7.5	21.4	15.2–26.9	<0.001*	1.00
Visual analog scale of abduction	41.7 ± 15.6	43.6	29.9–53.3	62.6 ± 15.1	68.1	59.4–71.4	<0.001*	1.00
Gait score	1.9 ± 0.3	1.8	1.8–2.0	2.1 ± 0.3	2.1	1.9–2.2	0.017	0.71
Udder firmness (kg)	2.9 ± 0.7	2.6	2.5–3.4	4.5 ± 0.9	4.5	4.1–5.0	<0.001*	1.00
Udder width (cm)	31.7 ± 3.9	31.3	30.3–33.9	38.0 ± 4.4	37.4	35.5–39.5	<0.001*	1.00
Hind limb distance (cm)	31.2 ± 5.9	31.5	30.1–33.5	38.9 ± 5.7	39.6	34.6–42.5	<0.001*	1.00
Cumulated depth of edema (mm)	0.0 ± 0.0	0.0	0.0–0.0	1.2 ± 1.5	0.6	0.0–1.9	<0.002*	0.80

[1]Mean of the 6 h preceding milking.

[2]Significance level = 0.002 (after Bonferroni correction); * if significantly different.

[3]Change between activities of eating, ruminating, and no activity.

[4]Movement of the limbs or <3 strides.

Table 2. Increase per hour (slope) of different variables from the early-lactation saline group (n = 15) for the baseline period and prolonged milking interval

Variable	Baseline period[1]			Prolonged milking interval[1]				
	Mean ± SD	Median	Interquartile range	Mean ± SD	Median	Interquartile range	*P*-value[2]	Power
Weight on hind limb (%/h)	0.10 ± 0.13	0.12	0.02 to 0.21	−0.03 ± 0.07	−0.04	−0.05 to 0.00	0.001*	0.95
Difference between front and hind limbs (kg/h)	−0.59 ± 0.87	−0.77	−1.20 to −0.06	0.20 ± 0.46	0.23	−0.02 to 0.34	0.002*	0.92
Visual analog scale of abduction (/h)	4.05 ± 3.32	3.50	2.00 to 5.54	1.01 ± 1.82	1.63	−0.14 to 1.87	0.006*	0.77
Udder firmness (kg/h)	0.23 ± 0.21	0.20	0.05 to 0.43	0.04 ± 0.21	0.02	−0.07 to 0.10	0.007*	0.75
Udder width (cm/h)	0.57 ± 0.70	0.33	0.11 to 1.08	0.52 ± 0.50	0.46	0.14 to 0.84	0.425	0.05
Hind limb distance (cm/h)	0.69 ± 1.43	0.65	−0.16 to 1.50	0.54 ± 0.81	0.40	0.01 to 0.94	0.350	0.06

[1]Increase per hour (slope) of the 6 h preceding milking.
[2]Significance level = 0.0071 (after Bonferroni correction); * if significantly different.

no differences in the duration of the incline and decline phases ($P = 0.116$ and $P = 0.060$, respectively; Table 3). The peak milk flow was not increased in the PMI ($P = 0.071$), but showed a trend to occur earlier in the BLP than in the PMI ($P = 0.010$; $\alpha = 0.006$; Table 3). The maximal electrical conductivity was 6.0 ± 0.6 mS/cm in the BLP and increased to 6.8 ± 0.9 mS/cm in the PMI ($P = 0.003$). The electrical conductivity during the incline phase was also increased in the PMI (Table 3). The SCC increased after the PMI and was increased in the milkings +12 h, +24 h, +48 h, and +72 h after the PMI ($P < 0.001$; Figure 3).

Bacteriological Culture. Major pathogens (*Streptococcus uberis*) were found in only 2/36 samples from before the PMI and 2/36 samples after. Only 1 cow was affected by *Streptococcus uberis* in experiments 1 and 2. We found minor pathogens in 22/36 and 19/36 of the milk samples before and after the PMI, respectively. The following minor bacteriological agents were successfully cultured: *Corynebacterium bovis* (17 and 11 samples before and after the PMI, respectively), coagulase-negative staphylococci (5 and 9 samples before and after the PMI, respectively), *Enterococcus avium* (2 and 2 samples before and after the PMI, respectively), *Streptococcus* spp. (2 and 2 samples before and after the PMI, respectively) and *Pantoea agglomerans* (0 and 1 sample before and after the PMI, respectively). The occurrence of bacteriological agents at different time points (before and after the PMI, only before the PMI or only after the PMI) is shown in Table 4.

Comparison of Baseline Period and Recovery Period. When comparing the BLP with the RP, we found a difference only in udder firmness, which was lower in the RP (3.0 ± 0.9 vs. 2.3 ± 0.7 kg; $P = 0.001$).

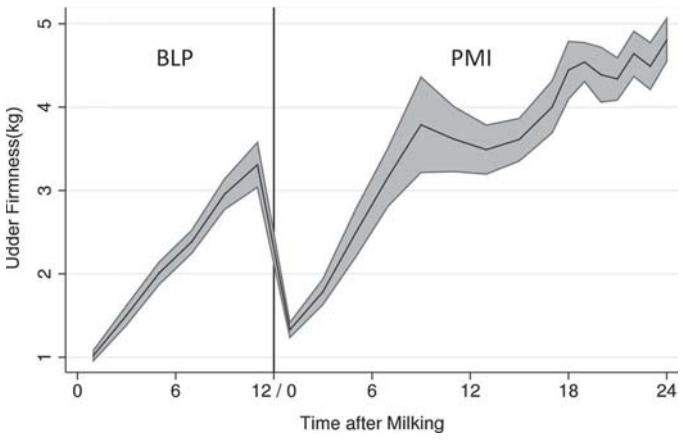

Figure 2. Trend of mean value of udder firmness (kg) during 12 h of baseline period (BLP) and 24 h of prolonged milking interval (PMI) in 15 cows from the early-lactation saline group. The gray shaded area represents the standard error.

All other variables were not significantly changed during the RP (data not shown).

Comparison of Groups

We found no significant difference between the early-lactation saline group and the flunixin or mid-lactation groups in any of the variables when comparing the values during the PMI directly against each other. From the early-lactation flunixin and mid-lactation groups, only those results that were contradictory with the results of the early-lactation saline group are reported. *P*-values for comparisons of the BLP with the PMI for all groups are given in Tables 5 and 6.

Early-Lactation Flunixin Group. In contrast to the early-lactation saline group, the occurrence and depth of edema was not different between the BLP and PMI (0/15 vs. 6/15, $P = 0.008$; and 0.0 ± 0.0 vs. 0.8 ± 2.4 mm, $P = 0.015$, $\alpha = 0.002$; respectively). We also found differences between the BLP and PMI in limb events and eating chews (87.4 ± 29.8 vs. 105.7 ± 28.2; $P < 0.002$; and $1{,}396 \pm 374$ vs. 987 ± 273/h; $P < 0.001$, respectively).

Mid-Lactation Saline Group. In the mid-lactation group, no variables of feeding behavior (eating time, eating chews, ruminating time, ruminating chews, and ruminating bolus) were different, when comparing the BLP with the PMI (*P*-values in Table 5). We found no differences in the absolute difference of weight placed on front and hind limbs (27.2 ± 6.1 vs. 23.6 ± 6.9 kg, $P = 0.003$; $\alpha = 0.002$) and in the VAS of abduction

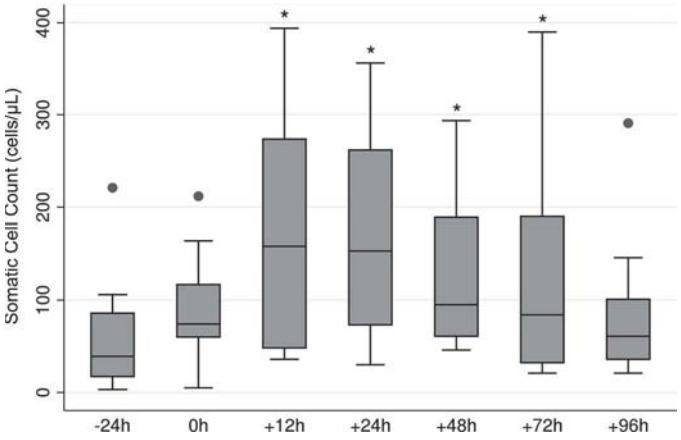

Figure 3. Box-plot representation of SCC at the end of the baseline period (−24 h), at the end of the prolonged milking interval (0 h) and for 4 d after the prolonged milking interval (+12 to +96 h) in 15 cows from the early-lactation saline group. *Significant increase compared with the baseline period (−24 h). The center line of the box represents the 50th percentile, the boundaries demarcate the 25th and 75th percentiles, and the whiskers include 1.5 × the interqartile range. The dots represent outliers.

Table 3. Milking data from early-lactation saline group (n = 15) for the baseline period and prolonged milking interval

	Baseline period[1]			Prolonged milking interval[1]				
Variable	Mean ± SD	Median	Interquartile range	Mean ± SD	Median	Interquartile range	*P*-value[2]	Power
Milk yield (kg)	14.4 ± 3.4	13.9	12.6–16.6	22.7 ± 6.4	22.5	17.4–25.0	<0.001*	1.00
Incline phase (min)	0.7 ± 0.3	0.7	0.5–0.8	0.5 ± 0.3	0.3	0.3–0.7	0.116	0.42
Plateau phase (min)	2.8 ± 1.9	2.5	1.5–4.4	4.9 ± 3.4	3.8	2.2–8.1	0.002*	1.00
Decline phase (min)	3.4 ± 1.7	3.1	2.0–5.0	4.3 ± 1.9	4.2	2.7–5.4	0.060	0.63
Time of peak milk flow (min)	2.6 ± 1.5	2.1	1.6–3.2	4.6 ± 3.3	2.7	2.1–8.0	0.010	0.78
Peak milk flow (kg/min)	3.8 ± 1.3	3.7	2.9–4.2	4.1 ± 1.3	3.9	2.9–5.1	0.071	0.42
Electrical conductivity incline (mS/cm)	6.0 ± 0.6	6.3	5.7–6.4	6.3 ± 0.8	6.6	5.6–6.9	0.004*	0.87
Maximal electrical conductivity (mS/cm)	6.0 ± 0.6	6.2	5.5–6.4	6.8 ± 0.9	6.7	5.8–7.2	0.003*	0.68

[1]Data of milking at the end of the period.
[2]Significance level = 0.006 (after Bonferroni correction); * if significantly different.

Table 4. Occurrence of the different bacteriological agents in milk samples collected before or after the prolonged milking interval, or both

Bacteriological agent	Before and after prolonged milking interval (no.)	Only before prolonged milking interval (no.)	Only after prolonged milking interval (no.)
Major pathogen			
Streptococcus uberis	2	0	0
Minor pathogen			
Corynebacterium bovis	8	9	3
Coagulase-negative staphylococci	5	0	4
Enterococcus avium	2	0	0
Streptococcus spp.	2	0	0
Pantoea agglomerans	0	0	1

(27.6 ± 7.6 vs. 52.9 ± 15.0; $P = 0.005$; $\alpha = 0.002$). Although limb distance was not different between the BLP and PMI ($P = 0.006$; $\alpha = 0.002$), udder width remained increased in the PMI ($P < 0.001$). Neither the occurrence nor the depth of edema was different between the BLP and PMI in mid lactation (1/10 vs. 6/10, $P = 0.029$; and 0.08 ± 0.08 vs. 0.66 ± 1.2 mm, $P = 0.186$, respectively).

DISCUSSION

Because the average milk yield per 305 d lactation in the Swiss Holstein population in 2014 was higher than in our study population (8,526 vs. 7,364 kg), further research is needed to estimate the effect of over-bagging in high-yielding cows. Most show cows are very high-yielding cows, so the impairment of well-being and health due to a PMI of 24 h might even be more distinct. The controlled and standardized settings and husbandry at the research station allowed us to evaluate the effect of a PMI on the health and well-being of dairy cows with minimal external disturbance (e.g., new animals in herd, stress due to the cow shows, changes in husbandry, and feeding). Because baseline data from each cow were used as their own controls, we did not test a separate control group.

Decreased feed intake (in kg/d) has been previously described for cows in pain because of mastitis (Fogsgaard et al., 2015) or lameness (González et al., 2008). The decrease in eating time in our study may also be explained by discomfort from increased intramammary pressure due to the over-bagged udder. The increased

Table 5. *P*-values[1] for the comparisons between mean values from the baseline period and prolonged milking interval for all 3 groups

Variable	Early-lactation saline group (n = 15)	Early-lactation flunixin group (n = 15)	Mid-lactation saline group (n = 10)
Eating time (min/h)	<0.001*	<0.001*	0.167
Eating chews (no./h)	0.013	<0.001*	0.440
Ruminating time (min/h)	<0.001*	<0.001*	0.017
Ruminating chews (no./h)	0.001*	0.001*	0.009
Ruminating bolus (no./h)	<0.001*	<0.001*	0.003
Chews per ruminating bolus (no.)	0.046	0.030	0.785
Change of feeding activity[2] (no./h)	0.003	0.013	0.121
Lying time (min)	0.231	0.035	0.196
Limb events[3] (no./h)	0.010	<0.002*	0.147
Stride duration (ms)	0.061	0.629	0.007
Stride distance (cm)	0.081	0.281	0.008
Relative weight on hind limb (%)	<0.001*	<0.001*	<0.002*
Difference between front and hind limbs (kg)	<0.001*	<0.001*	0.003
Visual analog scale of abduction	<0.001*	<0.001*	0.005
Gait score	0.017	0.056	0.021
Udder firmness (kg)	<0.001*	<0.001*	<0.002*
Udder width (cm)	<0.001*	<0.001*	<0.001*
Hind limb distance (cm)	<0.001*	<0.001*	0.006
Cumulated depth of edema (mm)	<0.002*	0.015	0.186

[1]Significance level = 0.002 (after Bonferroni correction); * if significantly different.

[2]Change between activities of eating, ruminating, and no activity.

[3]Movement of the limbs or <3 strides.

Table 6. *P*-values[1] for comparisons between increase per hour (slope) from the baseline period and prolonged milking interval for all 3 groups

Variable	Early-lactation saline group (n = 15)	Early-lactation flunixin group (n = 15)	Mid-lactation saline group (n = 10)
Relative weight on hind limb (%)	0.001*	<0.007*	0.001*
Difference between front and hind limbs (kg)	0.002*	0.012	0.004*
Visual analog scale of abduction	0.006*	0.008	0.444
Udder firmness (kg)	0.007*	0.006*	0.001*
Udder width (cm)	0.425	0.400	0.105
Hind limb distance (cm)	0.350	0.151	0.088

[1]Significance level = 0.007 (after Bonferroni correction); * if significantly different.

ruminating time we observed was unexpected, because ruminating is usually associated with good animal health (Soriani et al., 2012) and absence of acute stress (Herskin et al., 2004). In the context of this study, however, we might hypothesize that this represented a replacement activity because of decreased eating time. However, further research is needed to address this hypothesis.

Lying time is an indicator of welfare and has a high priority for dairy cows (Munksgaard et al., 2005). Although we found numerically decreased lying time in the PMI than in the BLP (8.5 ± 4.8 vs. 11.1 ± 5.5 min/h), this difference was not statistically significant ($P = 0.231$). In contrast to our findings, Österman and Redbo (2001) found increased standing time 4 h before milking in cows milked twice daily (11 to 15 h after milking) compared with cows milked 3 times per day (4 to 8 h after milking). Reduced lying time has also been found in cows with clinical mastitis (Fogsgaard et al., 2015). This difference from previous studies might be explained by the fact that our cows were examined during the observation phase (approximately 20 min/h or 2 h), and the cows in the other studies were not manipulated during the experiment. Additionally, the cows in our study were housed in a tiestall barn, and it is known that housing systems affect cow behavior, including lying behavior (Haley et al., 2000). Singh et al. (1994) found that healthy cows ruminated significantly longer during lying than during standing and stated that ruminating during lying was a sign of well-being in cows. In contrast, our cows showed a significant increase in ruminating time (13.3 ± 6.0 to 25.0 ± 2.8 min/h) and a numerical decrease in lying time (11.1 ± 5.5 to 8.5 ± 4.8 min/h) from the BLP to the PMI. Therefore, during the PMI, cows were ruminating most of the time while standing. This was a changed behavior compared with the BLP and could, therefore, indicate an impairment of their well-being. The fact that the cows in our study were examined every 1 or 2 h may have affected the results of cow behavior. But because the cows were habituated to the examination procedure and examined in the same way during the BLP and the PMI, we hypothesize that differences in behavior between the 2 groups were caused by the omitted milking and not by the examination itself or the change in examination frequency.

We found a weight shift to the hind limbs when comparing the BLP and PMI. This was in accordance with Chapinal et al. (2009), who found a decrease in weight on hind limbs immediately after milking compared with 1 h before milking. They explained this weight shift by the fact that 89% of milk is carried by the hind limbs. In contrast to weight distribution, we found no difference in the standard deviation of the weight taken per limb during the PMI, also supporting the results of Chapinal et al. (2009), who found no differences in standard deviation of weight per limb when comparing before and after milking. In contrast, cows with induced mastitis showed a decreased standard deviation per limb due to pain (Chapinal et al., 2013). Therefore, we assume that LPS-induced mastitis caused a more acute and distinctive pain response, reducing the movements of the hind limbs more than an over-bagged udder.

We found no difference in NRS scoring when comparing the BLP with the PMI. This was in accordance with Flower et al. (2006), who found no effect of milking on the NRS but in contrast to Chapinal et al. (2009) and Gleeson et al. (2007), who found an increase in locomotion score when comparing before and after milking and once- and twice-a-day milking, respectively. Chapinal et al. (2009) found only a slight decrease in NRS from before (NRS = 3.1) to after milking (NRS = 2.8), and Gleeson et al. (2007) used a lameness score, which included limb abduction. Because we found an increase in VAS of the hind limb abduction during the PMI, and Chapinal et al. (2009) found similar results, Gleeson's increase of lameness score might be explained by the fact that it included abduction.

The results of the subjective VAS of hind limb abduction were confirmed by objective measurements of the maximal hind limb distance and maximal udder width, which were both increased during the PMI and

showed a strong positive correlation with the VAS. The increased abduction of the hind limbs at standing and walking might be an attempt to avoid discomfort by decreasing pressure on the udder. Additionally, the increased VAS showed that the cows' gait during the PMI was impaired.

The udder firmness of cows milked once a day was significantly higher than that of cows milked twice a day when scored by manually palpating the udders (Gleeson et al., 2007). Our results of udder firmness assessed by the dynamometer during the PMI supported these findings and were in accordance with Bertulat et al. (2013). They found increased udder firmness in cows after abrupt drying off. As we assumed that no change in the firmness of the skin and udder tissue would occur during the experiment, we hypothesized that the increase in udder firmness might be caused by the increased amount of milk in the udder and, therefore, by increased intramammary pressure. The results of milk leaking supported this hypothesis, because all cows showed milk leaking during the PMI and increased intramammary pressure was shown to be a risk factor for milk leakage in an earlier study by Rovai et al. (2007). Also Bertulat et al. (2013) found an increased percentage of cows leaking with increased extramammary udder pressure due to drying off (56% in the high-yielding group). The higher percentage in our study might be explained by the stage of lactation (early vs. end lactation) and the higher milk yields of cows in our study (31.0 ± 7.0 vs. 17.6 ± 6.7 kg/d). Our results also confirmed the findings of Gleeson et al. (2007) and Tucker et al. (2007); both found an increased frequency of milk leaking in cows milked once a day compared with cows milked twice a day and in cows changed from twice-a-day to once-a-day milking, respectively.

The PMI had no effect on most variables of milk flow. However, milk yield and duration of the plateau phase were increased after PMI. The increased milk yield could be explained by the longer milking interval, because cows milked once a day have a higher milk yield in the milking following a PMI (Lakic et al., 2009). The prolonged plateau phase was a consequence of increased milk yield, because milk yield is correlated with duration of plateau phase (Weiss et al., 2004).

Electrical conductivity was increased at first milking after the PMI. Increases in electrical conductivity are caused by an influx of electrolytes through a damaged blood-milk barrier (Bruckmaier et al., 2004). Stelwagen et al. (1997) found that tight junctions in the blood-milk barrier change to a leaky state after 18 h of milk stasis, explaining the increase of electrical conductivity we found in the milking following the PMI. According to Stelwagen et al. (1997), this is a reversible phenomenon, because the blood-milk barrier closes again shortly after milking, explaining the decline in electrical conductivity to a normal level we observed at the second milking after PMI.

Leakage of the blood-milk barrier might also be related to the edema we found during the PMI. We detected the first signs of edema after 18h of milk stasis, concordant with the change of the blood-milk barrier to a leaky state (Stelwagen et al., 1997). After 24 h of milk stasis, we detected edema in 10/15 cows in the early-lactation saline group. The mean time after milking until detection of edema for all affected cows was 21.3 h, comparable to the 19 h observed in cows screened for edema due to over-bagging at a dairy cow show (Waller et al., 2007). Edema occurs rather late in the PMI compared with changes in udder firmness or weight distribution, because these variables formed a plateau after 18 h of milking, and the edema was on average first detected 21 h after milking. Both increased electrical conductivity and edema in the subcutaneous tissue indicate non-physiological processes toward the end of a 24-h milking interval.

In our study, the mean SCC increased from 66.3 cells/μL in the BLP to 216.3 cells/μL in the milking 12 h after PMI. A similar increase after a single PMI was also reported by Lakic et al. (2009, 2011). All assessed cows returned to a physiological concentration of SCC without treatment within 1 wk after the PMI, indicating that the increased SCC was an inflammatory reaction caused by the PMI, not by an IMI. This assumption was confirmed by the results of bacteriological cultures: we found 1 major pathogen 12 h after the PMI in only 1 cow, and this infection was already present before the PMI. We did not analyze the long-term effect of a PMI on udder health in this study, but it should be investigated thoroughly in further studies. The fact that the SCC exceeded physiological limits after a single PMI represents a non-physiological process during the lactation period. We found no increase in superficial udder temperature, as was found in cows with subclinical mastitis (Colak et al., 2008; Polat et al., 2010). These findings again confirm our assumption that the increase in SCC in our study was not caused by an IMI. We also found no increase in bacteriological colonization of the udder caused by major pathogens after the PMI, compared with before the PMI. As well, we found no increase in the number of minor pathogens after the PMI. However, the incidence of CNS increased from 4/36 to 9/36 and the incidence of *C. bovis* decreased from 18/36 to 11/36 from before to after the PMI. However, the incidence of CNS might have been overestimated, because freezing milk samples increases detection of these bacteria (Schukken et al., 1989).

Salivary cortisol concentration was not increased during the PMI in our study. This was in accordance with Tucker et al. (2007), who found no increase in fecal glucocorticoid concentration in cows changed from twice- to once-a-day milking in mid-lactation. However, Bertulat et al. (2013) found increased fecal glucocorticoid concentration after abrupt drying off in high-yielding cows. However, cows in that study also experienced changes in barn, group, and diet at drying off, which might have affected the results.

In different variables (e.g., weight distribution, udder firmness, VAS of abduction), we detected a higher slope of the respective variable during the last 6 h of the BLP compared with the last 6 h of the PMI, and the slopes in the PMI were close to zero, showing a plateau formation. Milk production per hour decreased after 16 h of milk accumulation in the udder, compared with the constant milk production before 16 h (Davis et al., 1998). Therefore, plateau formation can most likely be explained by the fact that milk production was lower during the last 6 h of the PMI.

Most results from the flunixin group were comparable to the results from the early-lactation saline group. We concluded that a single dose of flunixin administered at the beginning of the PMI, as is occasionally practiced during the preparation of cows for Swiss dairy cow shows, only slightly improved animal well-being during a PMI of 24 h, because only the formation of udder edema was decreased in the flunixin group. The plasma half-life of flunixin is approximately 4 h (Kissell et al., 2012). Therefore, the effect of flunixin might have been more prominent if flunixin was administered later in the experiment. In contrast, results from the mid-lactation group showed that a milk stasis of 24 h in mid-lactation had less effect on well-being of cows, indicating that the threshold of a PMI for causing discomfort and signs of disease in individual cows is modified by the stage of lactation. Therefore, it is not feasible to simply limit the maximal duration of a PMI allowed at dairy cow shows by a defined number of hours to increase animal welfare. However, we can assume that a milk stasis of over 18 h compromises the well-being of show cattle. Furthermore, cows at a dairy show are exposed to additional changes in feeding, housing, and composition of groups. Further research is needed to assess to what degree the well-being of cows during a PMI at a dairy show is compromised and which measurable clinical signs are useful for detecting cows with decreased well-being due to exceeded udder fill.

CONCLUSIONS

We conclude that the well-being of cows during a PMI of 24 h is disturbed, because we found changes in behavior, increased udder firmness, weight shifting from front to hind limbs, and increased abduction of the hind limbs. Cows also underwent non-physiological processes: we found edema in the subcutaneous udder tissue (late in the PMI), increased milk leaking, and non-physiologically increased SCC.

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