The effect of individual quarter dry-off in management of subclinical mastitis on udder condition and milk production in organic dairy herds: A randomized field trial

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ABSTRACT

The objective of the study was to evaluate the effect of individual quarter dry-off in management of subclinical mastitis on clinical development, behavior during milking, and short-term production loss. The study was conducted as a controlled randomized trial in 5 commercial organic dairy herds. Seventy cows meeting the inclusion criteria were randomly allocated to 1 of 2 treatments: individual quarter dry-off (QDO) or continued milking. The inclusion criteria constituted an increase in somatic cell count from below to above 400,000 cells/mL at milk recording and identification of a quarter scoring of at least 3 and higher than the remaining quarters at California mastitis testing (scale 1–5). Quarters were evaluated clinically for atrophy, swelling, firmness, signs of pain, and milk leakage, and rectal temperature of the cows was measured. Behavior during milking was assessed as average frequency of hind limb tripping and kicking during milking obtained by registrations on video recordings. Milk production loss was estimated based on average daily milk yield for 19 d subsequent to treatment start, with reference to average daily milk yield in 19 d before treatment. The QDO treatment was associated with quarter swelling and increased quarter firmness around d 10 from treatment start and with quarter atrophy around d 40 from treatment start. However, around d 40 from treatment start 35% of the dried-off quarters remained non-atrophic and only 6% of the quarters were completely dry. The QDO was associated with signs of pain related to the dried-off quarter. No obvious effects on behavior during milking of QDO treatment was observed. On average the production loss associated with QDO was 4.1 kg/d (95% confidence interval: 3.1–5.0) greater than for continued milking. The production loss depended on parity, days in milk, and prior yield on the quarter sub-

jected to dry-off (Q1). The production loss increased with increasing prior yield on Q1. For the majority of cows the increase in production loss was below 1 kg of milk/d when the prior yield on Q1 increased by 1 kg of milk/d, demonstrating a compensatory production potential of the nontreated quarters. Early lactation multiparous cows showed the greatest compensatory potential. Further studies into the effect on welfare, cure rates, and transmission are needed to determine the feasibility of individual quarter dry-off.

Key words: quarter dry-off, subclinical mastitis, organic dairy cow, yield

INTRODUCTION

Mastitis in dairy cattle is the inflammation of one or more quarters of the udder. The etiology is predominantly bacterial pathogens ascending through the teat canal (Quinn, 2011). Mastitis may be classified as clinical if visible inflammatory signs or alterations in the milk are present. Alternatively, mastitis may be subclinical, where the inflammatory response is only detectable by laboratory procedures. Such inflammatory responses include elevated SCC in the milk resulting from an increase of leukocytes in the udder gland (Erskine, 1992). In addition to reduced milk quality, subclinical mastitis is associated with a reduced milk production, thus causing economic losses for the dairy producer (Halasa et al., 2009).

Antibiotic treatment of subclinical mastitis is often omitted as the costs associated with treatment and milk detained from delivery due to antibiotic residues seem extensive compared with the effect of the disease (Swinkels et al., 2005). Additionally, restrictions on use of antibiotics to limit the selection of resistant pathogens further opposes the choice of antibiotic treatment. This is of special relevance in organic dairy production. Limited use of antibiotics is a separate goal in organic farming balanced against promotion of animal health and welfare, as reflected in the statutory restrictions [Council Regulation (EC) No 834/2007, 2007]. However, further considerations in leaving subclinical

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mastitis untreated include the continual risk of clinical episodes and transmission of contagious pathogens to other cows, which may in turn result in more mastitis cases that require antibiotic treatment. This fuels the interest for non-antimicrobial management strategies for subclinical mastitis in line with the current interest regarding clinical mastitis (Francoz et al., 2017).

A cross-sectional study indicated that approximately 10% of lactating dairy cows in Danish organic herds had at least one dry quarter (discontinued milking of one udder quarter during lactation) (Bennedsgaard et al., 2010). Some quarters are nonfunctional at first calving (Duraes et al., 1982). However, surveys among Danish organic dairy farmers showed that drying off quarters was practiced as a strategy for handling recurrent mastitis and chronically elevated SCC (Vaarst et al., 2006; Hansen and Sørensen, 2017). Drying off quarters could be an alternative to antibiotic treatment of subclinical mastitis with the benefits of reduced risk of transmission of mastitis pathogens to other cows and reduced amount of infected milk into the bulk tank (Leitner et al., 2012). However, limited knowledge is available on the feasibility and consequences of the procedure. Studies indicate that drying off a quarter is associated with a compensatory milk yield increase in adjacent quarters, suggesting that the production loss associated with the procedure may be limited (Hamann and Reichmuth, 1990). However, drying off individual quarters may cause pain or discomfort due to tension from the retained milk (Bertulat et al., 2013) and may increase the risk of developing clinical mastitis. The objective of this study was to investigate the effect of quarter dry-off (**QDO**) during lactation compared with continued milking (**CM**) on clinical development, behavior during milking, and short-term milk production.

MATERIALS AND METHODS

The study was conducted from August 2016 to November 2017 as an experimental study with a parallel group design. Organic dairy cows were randomly allocated to 1 of 2 treatments: QDO or CM with an allocation ratio of 1:1.

Herds

Five commercial Danish organic dairy herds were recruited for the study. For recruitment, letters were sent to potential study herds, identified as commercial organic dairy herds with automatic milking system (**AMS**), geographically located within 200 km of the research institute. By phone interviews, starting at the larger herds, the farmer's willingness to participate was assessed until 4 participants were identified. An addi-

tional herd was recruited 4 mo into the study period to increase the number of cows in the study.

Four of the participating herds (A, B, D, and E) consisted of Danish Holstein cows, and 1 herd (C) consisted of Jersey cows. Herd size ranged from 131 to 200 cow-years. The AMS were either DeLaval (B, D, and E) or Lely (A and C). Milk yield recordings were done 11 times annually.

Cows

Cows for the study were sampled from lactating cows being at least 30 d postpartum, at least 60 d before expected dry-off and at least 30 d from last mastitis treatment. At each milk recording cows were identified as potential study cows based on their recorded SCC. The criterion was a SCC above 400,000 cells/mL at the current milk recording and below 400,000 cells/mL at the preceding recording. The cut-off value for SCC of 400,000 cells/mL was used to ensure the practical relevance of the dry-off procedure and provide a strict inclusion criterion. A second criterion was a total yield of less than 40 kg of milk within the last 24 h based on data from the AMS. As QDO was expected to cause distension on the subjected quarter to a degree related to the production of the cow, the upper yield limit was instituted to restrict the degree of distention. Finally, cows with blind quarters were excluded as well as cows exhibiting signs of severe diseases such as pneumonia. Additionally, cows with signs of severe mastitis were excluded, whereas cows the mild clinical signs of mastitis were not excluded. Cows meeting the criteria were randomly allocated to QDO or CM using the RANUNI function in SAS 9.4 (SAS Institute Inc., Cary, NC) with a seed value of zero and a dichotomization threshold of 0.5. To ensure that the case of subclinical or mild clinical mastitis could be related to a specific quarter, the study cows were subsequently tested with the California Mastitis Test (**CMT**; Levesque, 2004). This was done within 5 to 36 (median 13) days from milk recording. A scale of 1–5 was used (1: mixture remains liquid, 2: reversible thickening, 3: persistent thickening but no gel forming, 4: gel forming, 5: sticking gel). If the CMT score was at least 3 on one quarter (**Q1**) and the remaining quarters (**Q3**) scored at least one CMT-score value less than the identified quarter, then the cow was included in the study. A CMT score of at least 3 is associated with a SCC of above 400,000 cells/mL. The cut-off value for SCC of above 400,000 cells/mL at the last milking recording was further used to select toward cows for which identification of a quarter with a CMT score of at least 3 could be expected. The quarter (**Q1**) identified with the highest CMT score was the quarter that was dried off in cows allocated to QDO.

In total 84 cows met the inclusion criteria and initiated treatment. Of these, 14 were subsequently excluded for unforeseen reasons. Some were culled ($n = 5$), dried ($n = 1$), or sold ($n = 1$) before follow-up. For 7 cows the farmers restarted milking on the quarter subjected to QDO after a few days. Two reasons were given for this; one farmer restarted milking on 4 cows, due to an observed yield drop at treatment start, which he found uncomfortably large and another farmer restarted milking on 3 cows due to confusion related to treatment allocation. The remaining 70 cows were included in the study. For 60 cows treatment was in agreement with the allocation protocol, whereas 10 cows received the treatment opposite from the one to which they were randomly allocated. Five of these were allocated to CM, but received QDO, whereas 5 were allocated to QDO, but received CM. In these cases the farmers failed to consult the individual cow allocations and instead subjected all cows included at the current milk recording to the same treatment. These cows were not omitted, as the group allocation was estimated to be as random as the individual allocation.

Quarter Dry-Off Procedures

The procedure for QDO was chosen and performed by the farmer. Complete cessation of milking of the quarter after 7 d was required by all farmers. In herds A and E quarters were dried off by abrupt cessation of milking of the quarter (abrupt QDO). In the remaining herds the quarter was milked once or twice during the week following treatment start (gradual QDO). Milking was done on d 3 or 4 in the AMS in herd B, on d 3 and 6 by hand in herd C and on d 1 and 6 in the AMS in herd D.

Milk Samples

Quarter level milk samples were collected by the farmer from the quarter identified with highest CMT-score on the day of enrollment, d 0. At the start of the study, the farmers were instructed in an aseptic sampling protocol. Milk samples were stored at -18°C until bacterial culture was performed. At culture, milk samples were plated on blood agar plates with esculin (Eurofins, Vejen, Denmark, and Thermo Fisher Scientific, Waltham, MA) and chrome agar plates (Becton Dickinson, Franklin Lakes, NJ). Plates were incubated aerobically at 37°C and read at 24 and 48 h. Microbial species were identified based on colony morphology, hemolysis pattern, esculin hydrolysis, catalase test reaction, and microscopy. Suspected *Staphylococcus aureus* were confirmed on Vogel-Johnson agar, whereas suspected *Streptococcus uberis* were differentiated from

Enterococcus spp. on sodium-chloride enriched tryptic soy agar (9%). Quarters were classified as infected if a pure culture of more than 2 cfu was observed. Samples were classified as contaminated if more than 2 species were identified at culture.

Clinical Observations

All 4 quarters of the study cows were assessed by the farmer at d 0, 1, 3, and 7 following treatment allocation of the cow. At study start, each farmer was instructed in the assessment, to facilitate agreement between farmers. The farmers inspected and palpated the quarters to assess atrophy, swelling, firmness, milk leakage, and signs of pain. Firmness was assessed as increased firmness compared with the remaining quarters, whereas milk leakage was assessed as milk dripping or running from the nonmanipulated quarter with no leakage from the remaining quarters. Signs of pain were assessed as avoidance behavior, kicking, or back arching at palpation. All observations were registered as present (1) or absent (0). Signs of pain were registered as present if one sign was observed. Equivalent clinical assessment of the quarters of each cow was conducted by the first author twice: once at d 7 to 14 (d 10) from enrollment and once at d 30 to 60 (d 40) from enrollment. Assessment at d 0 and 40 of the quarter with the highest CMT score further included observations for precipitations in the milk, such as flakes or clots. Additionally, rectal temperature was measured by the farmer at d 0, 1, 3, and 7 from treatment start. Six cows were not examined on d 1 to 7 after treatment start, whereas 4 cows were not examined on d 10.

Behavior During Milking

The behavior of the cows during milking was recorded in 3 herds (A, C, and D) by video cameras. The cameras were set up to visualize the rear part of the cows. S-VIDIA Client MegaPixel 6.0 (S-VIDIA, Tacoma, WA) was used to read the recordings. Two observers made registrations on the behavior following the ethogram provided by Fogsgaard et al. (2015a). A hoof lift less than 15 cm off the ground was classified as a trip, and a lift of more than 15 cm was classified as a kick (Rushen et al., 2001). Tripping and kicking were recorded on the hind leg closest to the camera, as lifting height could not be assessed on the remote leg. Registrations were done for the first milking on d -6 , -3 , and -1 before enrollment and d 1, 3, 6, and 9 after enrollment. If the first milking of the day was not observable due to blocking of the camera view by other cows, registrations were done on the subsequent milking. The observers noted the duration of the observed

milking and the duration of periods within the milking in which camera view was blocked.

Milk Production

Data on quarter milk production at each milking were obtained from the AMS. Data on calving dates were obtained from the Danish cattle database.

Statistical Analysis

Rectal temperature was dichotomized into fever and nonfever using a threshold value of 39.4°C (Adams et al., 2013). Association between clinical outcomes and treatment were evaluated by Fisher exact test using Benjamini and Yekutieli (2001) correction at multiple comparisons. Odds ratios and 95% confidence intervals for these were obtained through the Fisher exact test. The same method was used for evaluation of clinical outcomes against QDO procedures.

Milk yield before treatment start was calculated as the average daily yield in the period from d -19 to -1 before treatment start. The posterior milk yield following treatment start was calculated as the average daily yield in the period from d +8 to +26. The production loss was calculated as the difference between the prior and the posterior yield. The prior quarter yield on Q1 was calculated as the average daily yield on Q1 in the period from d -19 to -1 before treatment start. In each case, milk yield was averaged over a period of 19 d to ensure that the result was not heavily influenced by 1 or 2 abnormal milkings, but was still representative for the time point of interest.

The univariable effect of treatment, herd, breed, and parity on production loss was evaluated using ANOVA. For pairwise comparison of herds, the *t*-test was used adjusting with the Benjamini and Yekutieli (2001) method. Linear regression was used for the effect of DIM, total prior yield, and prior quarter yield of Q1. Days in milk ranged from 70 to 548 d. The cows were unevenly distributed on the range of DIM, as only 7 (10%) cows were more than 300 d from calving. Thus, these were excluded from the multivariable analysis. All independent variables were included in a multivariable ANOVA. However, herd and breed were not included simultaneously, but evaluated separately along with the remaining variables. Herd was included as a fixed variable, as the 5 study herds were not expected to represent a random sample of Danish organic dairy herds. A full model with the 3-way interaction between DIM, prior yield on Q1, and treatment groups, all 2-way interactions, and main effects was created. Model reduction was carried out by backward elimina-

tion based on the *F*-test and model control was done by assessment of residual plots. Two observations with large standardized residuals were excluded from the analyses on production loss and the multivariable model was refitted accordingly. To investigate the effect of the different QDO procedures on production loss, the procedures were introduced into the multivariable model with the highest adjusted R^2 . Similarly, the variables of precipitates in milk, quarter swelling, and signs of pain at d 0 were introduced into the obtained multivariable model to investigate the effect of clinical signs at treatment start on production loss.

To check for collinearity between prior total yield and prior quarter yield on Q1, the Pearson correlation coefficient for these was calculated. The obtained coefficient did not exceed 0.8 for highly correlated variables.

The frequency of tripping and kicking per minute before treatment start was calculated as the average frequency of the 3 observed milkings (d -6, -3, and -1) divided by the observation time. Observation time constituted the duration of the observed milkings subtracted time periods where the camera view was blocked. Similarly, the frequency of tripping and kicking per minute after treatment start was obtained by averaging registrations from the 4 observed milkings (d +1, +3, +6, and +9).

Statistical analyses were performed in R 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria) and test results were evaluated at a 0.05 significance level.

RESULTS

Population

Of the 70 cows included in the study, 36 cows received CM, whereas 34 received QDO. Of the 34 cows subjected to QDO, 11 had abrupt QDO whereas the remaining 23 had gradual QDO. The distribution of herd, breed, parity, DIM, prior total yield, and prior quarter yield of Q1 on treatments are given in Table 1.

Bacterial Culture Results

Culture results are shown in Table 1. For 20 (29%) milk samples, no growth was obtained at culture. The most frequently isolated pathogens constituted *Streptococcus uberis* (18%), *Staphylococcus aureus* (16%), and CNS (20%), whereas *Streptococcus dysgalactiae* (7%) and *Escherichia coli* (3%) constituted a minority of the infections. Cows subjected to CM contributed the majority (85%) of *S. uberis* isolates, whereas cows subjected to QDO contributed with a minority (15%).

Clinical Development

Results for the clinical outcomes are shown in Table 2. In the week following treatment start (d 1–7), 13% of the cows receiving QDO had a temperature of above 39.4°C at least once. In comparison, 3% of the cows receiving CM had a temperature of above 39.4°C. However, the observed difference in rectal temperature on treatment was nonsignificant ($P = 0.19$).

At the final examination on d 40 the prevalence of atrophic quarters had increased in both treatment groups since treatment start. The increase was greater among QDO cows with 22 (65%) atrophic quarters, compared with 15 (42%) among CM cows. However, the difference between the treatments at d 40 was not significant ($P = 0.06$).

At d 10 the prevalence of swollen quarters had increased since treatment start, so that the prevalence was higher for QDO cows than for CM cows ($P = 0.01$). The odds for quarter swelling among cows receiving QDO was 3.8 (95% CI: 1.3–13) times greater than for cows receiving CM. A similar increase at d 10 was observed for increased quarter firmness, with a significant ($P = 0.01$) difference between the treatments. At d 40 the prevalence of quarter swelling had decreased to a level approximating that observed at treatment

start and no significant difference between the treatments was observable. Likewise, a significant difference between the treatments was not apparent at d 40 for increased quarter firmness, as the prevalence decreased slightly among QDO cows, while a further increase was observed among CM cows.

Following treatment, in the period from d 1 to the last examination at around d 40, in total 10 (29%) cows subjected to QDO were registered with signs of pain at least once, whereas that was the case for 3 (8%) cows subjected to CM. This difference was significant ($P = 0.03$), with the odds for signs of pain being 4.5 times greater among QDO cows compared with CM cows. Of the 10 QDO cows registered with signs of pain related to Q1, 4 presented in the week following treatment start (d 1, 3, or 7) and 4 presented on d 10. The remaining 2 were only observed with signs of pain on the last examination (d 40). The prevalence of signs of pain related to Q3 (quarters not subjected to treatment) was also greater among QDO cows than CM cows. However, this difference was not significant ($P = 0.11$).

In the week following treatment start milk leakage was observed in 9 (29%) of the QDO cows, whereas this was not noted for any CM cows, thus corresponding to a significant ($P < 0.001$) difference between treatments. For 2 QDO cows milk leakage was only noted on d 1

Table 1. Descriptive statistics of herd characteristics, milk production, and bacterial culture for cows with elevated SCC subjected to continued milking (CM) and individual quarter dry-off (QDO)

Variable	Level	Total	Treatment	
			CM	QDO
Enrolled cows ¹		70 (100)	36 (51)	34 (49)
Herd ¹	A	23 (33)	16 (70)	7 (30)
	B	14 (20)	3 (21)	11 (79)
	C	19 (27)	10 (53)	9 (47)
	D	9 (13)	6 (67)	3 (33)
	E	5 (7)	1 (20)	4 (80)
Breed ¹	Holstein	51 (73)	26 (51)	25 (49)
	Jersey	19 (27)	10 (53)	9 (47)
Parity ¹	1	19 (27)	8 (42)	11 (58)
	≥2	51 (73)	28 (55)	23 (45)
DIM ²		170 (70–548)	174 (70–478)	169 (72–548)
Prior yield ² (kg/d)		29.6 (11.7–46.2)	31.0 (11.7–46.2)	27.7 (15.7–43.4)
Prior quarter yield on Q1 ^{2,3} (kg/d)		6.4 (1.4–11.1)	6.5 (1.4–11.1)	6.2 (2.8–10.8)
Culture result (Q1) ^{1,4}				
No growth		20 (29)	10 (50)	10 (50)
<i>Streptococcus uberis</i>		13 (18)	11 (85)	2 (15)
<i>Staphylococcus aureus</i>		11 (16)	6 (55)	5 (45)
CNS		14 (20)	6 (43)	8 (57)
<i>Streptococcus dysgalactiae</i>		5 (7)	1 (20)	4 (80)
<i>Escherichia coli</i>		2 (3)	1 (50)	1 (50)
<i>Enterococcus</i> spp.		1 (1)	0	1 (100)
Contaminated		4 (6)	1 (25)	3 (75)

¹No. (%), column-wise percent for totals and row-wise percent for treatment groups.

²Median (minimum–maximum).

³Q1 = quarter identified with highest California Mastitis Test score.

⁴Culture results for milk samples taken at the day of enrollment.

following treatment start, whereas 3 had milk leakage until d 3 and 4 had milk leakage until d 7 following treatment start. At d 10 following treatment start, an additional 3 cows were noted with milk leakage and one of these also showed milk leakage on d 40 following treatment start.

Treatment for mastitis with antibiotics after enrollment but within the current lactation were registered for 3 study cows. Two CM cows had antibiotic treat-

ment for mastitis 3 and 62 d after study enrollment, respectively, whereas one QDO cow had antibiotic treatment for mastitis 85 d after QDO.

For the results on clinical outcomes shown in Table 2, QDO was evaluated collectively without any differentiation in relation to the procedure used. Clinical outcomes were further evaluated against the 2 different QDO procedures: abrupt QDO as used in herd A and E and gradual QDO as used in herd B, C, and D. The

Table 2. Effect of continued milking (CM) and individual quarter dry-off (QDO) on clinical observations

Day ¹	n (%) ²		Treatment [no. (%) ³]		OR ⁴ (95% CI)	P-value
			CM	QDO		
Total	70 (100)		36 (51)	34 (49)		
Rectal temperature (°C)						
0	70 (100)	≤39.4	36 (51)	34 (49)	0 (0–Inf ⁵)	1
		>39.4	0	0		
1–7	64 (91)	≤39.4	32 (97)	27 (87)	4.6 (0.4–240)	0.19
		>39.4	1 (3)	4 (13)		
Precipitates in milk						
0	70 (100)	Non	28 (78)	29 (85)	0.6 (0.1–2.4)	0.54
		Present	8 (22)	5 (15)		
40	70 (100)	Non	33 (92)	21 (62)	5.6 (1.3–35)	0.04 ⁶
		Present	3 (8)	11 (32)		
		Dry	0	2 (6)		
Gland atrophy						
0	70 (100)	Non	30 (83)	25 (74)	1.8 (0.5–7.0)	0.39
		Present	6 (17)	9 (26)		
40	70 (100)	Non	21 (58)	12 (35)	2.5 (0.9–7.6)	0.06
		Present	15 (42)	22 (65)		
Gland swelling						
0	70 (100)	Non	34 (94)	28 (82)	3.6 (0.6–39)	0.15
		Present	2 (6)	6 (18)		
10	66 (94)	Non	25 (71)	12 (39)	3.8 (1.3–13)	0.01
		Present	10 (29)	19 (61)		
40	70 (100)	Non	30 (83)	30 (88)	0.7 (0.1–3.2)	0.74
		Present	6 (17)	4 (12)		
Gland firmness						
0	70 (100)	Normal	35 (97)	34 (100)	0 (0–41)	1
		Increased	1 (3)	0		
10	66 (94)	Normal	31 (89)	18 (58)	5.4 (1.4–26)	0.01
		Increased	4 (11)	13 (42)		
40	70 (100)	Normal	28 (78)	22 (65)	1.8 (0.6–6.4)	0.29
		Increased	8 (22)	12 (35)		
Signs of pain Q1 ⁷						
0	70 (100)	Non	36 (100)	33 (97)	Inf (0.03–Inf)	0.49
		Present	0	1 (3)		
1–40	70 (100)	Non	33 (92)	24 (71)	4.5 (1.01–28)	0.03
		Present	3 (8)	10 (29)		
Signs of pain Q3 ⁸						
0	70 (100)	Non	36 (100)	34 (100)	0 (0–Inf)	1
		Present	0	0		
1–40	70 (100)	Non	36 (100)	31 (91)	Inf (0.4–Inf)	0.11
		Present	0	3 (9)		

¹Days from treatment start.

²Column-wise percent.

³Row-wise percent.

⁴OR: odds ratio estimates for QDO against CM (Fisher's exact test).

⁵Inf = infinity.

⁶P-value for test of no precipitates in milk against presence of precipitates in milk.

⁷Q1 = quarter subjected to treatment.

⁸Q3 = quarters not subjected to treatment.

Table 3. Univariable analyses on production loss (kg/d) for cows with elevated SCC subjected to either continued milking (CM) or individual quarter dry-off (QDO)

Variable	Level	Estimate	SE	95% CI	<i>P</i> -value
Treatment	CM	1.6	0.3	0.9; 2.2	<0.001
	QDO	5.6	0.5	4.9; 6.3	
Herd	A	2.8	0.6	1.6; 3.9	
	B	5.3	1.0	3.8; 6.8	
	C	3.4	0.9	2.2; 4.7	
	D	2.9	1.1	0.9; 4.8	
	E	4.0	1.4	1.5; 6.4	0.10 ¹
Race	Holstein	3.6	0.4	2.8; 4.4	
	Jersey	3.4	0.8	2.1; 4.7	0.82
Parity	1	3.5	0.7	2.1; 4.9	
	≥2	3.5	0.8	2.8; 4.3	0.95
DIM (cont. ²)		−0.004	0.003	−0.011; 0.003	
Prior yield (kg/d; cont.)		0.03	0.04	−0.06; 0.12	
Prior yield on Q1 ³ (kg/d; cont.)		0.19	0.15	−0.11; 0.49	

¹Lowest *P*-value obtained at pairwise comparison, herd A compared with herd B.

²cont. = continuous variable.

³Q1 = quarter subjected to treatment.

prevalence among gradual QDO cows of gland swelling (70%) and increased gland firmness (45%) at d 10 and signs of pain for Q1 (35%) at d 1 to 40 was greater than the corresponding prevalence among abrupt QDO cows of 45, 36, and 18% for swelling, firmness, and pain, respectively. However, no significant differences between the 2 procedures with regard to the clinical outcomes were observable. Similarly there was no difference between the 2 procedures ($P = 1$) for milk leakage in the week following treatment start, as this was observed for 6 (30%) abrupt QDO cows and 3 (27%) gradual QDO cows.

Behavior During Milking

Video recordings of milkings were available for 23 cows of which 8 received QDO. Before treatment start, the median frequency of tripping was 0.9 (IQR 1.7) for CM cows and 1.1 (IQR 0.6) for QDO cows, whereas the median frequency of kicking was 0.2 (IQR 0.5) for CM cows and 0.1 (IQR 0.2) for QDO cows. After treatment start, the median frequency of tripping was 1.0 (IQR 1.3) for CM cows and 0.8 (IQR 1.0) for QDO cows, whereas the median frequency of kicking was 0 (IQR 0.3) for CM cows and 0.1 (IQR 0.2) for QDO cows. For the majority of cows regardless of treatment [11 (73%) CM, 6 (75%) QDO], a decrease in frequency of trips per minute was observed from before to after treatment start. This was also the case for the frequency of kicks per minute.

Milk Production Loss

At univariable analysis (Table 3) the short-term production loss was significantly ($P < 0.001$) different be-

tween the treatments. Two observations were identified with large standardized residuals. One of these received QDO and had a production loss of 13.4 kg/d, whereas the other received CM and had a production loss of 12.4 kg/d. These losses exceeded those of the remaining cows that ranged from −2.8 to 10.1 kg/d.

Two different multivariable models resulted when either herd or breed was included in model building, although herd and breed were both removed at model reduction. The model with the highest adjusted R^2 (0.68) resulted at inclusion of herd in model building and estimates from this are presented in Table 4. The model including breed in model building deviated from this in 2 aspects; parity did not show an interaction with prior yield on Q1 and the interaction with DIM related to total prior yield instead of prior yield on Q1. When herd was included in model building as a random variable, an interclass correlation of 0.002 was obtained.

For the study population used in multivariable analyses DIM ranged from 70 to 278, whereas prior Q1 yield ranged from 1.4 to 11.1 kg/d. Production losses predicted by the model within these ranges are displayed in Figure 1. Figure 1 shows the predicted production loss plotted against prior yield on Q1 for primiparous and multiparous cows receiving either CM or QDO. For each group predictions are plotted for cows either 70, 175, or 280 DIM at treatment start. First, consider the results for the CM cows representing cows with elevated SCC. For both primiparous and multiparous cows production loss decreased with increasing DIM at treatment start. The effect of DIM was more pronounced at lower prior Q1 yield. In general, primiparous cows experienced lower production losses than multiparous cows. However, at higher Q1 yield the

production losses for primiparous cows exceeded that of multiparous cows, resulting from a greater general dependence on prior Q1 yield for the primiparous cows compared with multiparous cows.

Considering the QDO cows, an increase in production loss with increasing prior Q1 yield was apparent regardless of parity and DIM at treatment start. For primiparous QDO cows the association between production loss and prior Q1 yield ranged from 0.75 at 70 DIM to 1.6 at 280 DIM based on the estimates in Table 4. Thus, an increase in prior Q1 yield of 1 kg/d was associated with an increased production loss of between 0.75 and 1.6 kg/d, with the lower values pertaining to cows shorter from calving. Primiparous cows at 133 DIM constituted the threshold for the association being below or above 1. For multiparous QDO cows the increase in production loss at each unit increase in prior Q1 yield ranged from 0.18 kg/d at 70 DIM to 1.0 kg/d at 280 DIM. Thus, for multiparous cows and primiparous cows less than 133 d from calving the increase in production loss was expected to be lower than the increase in prior yield on the dried-off quarter.

A multiparous cow 70 d from calving with an average daily Q1 yield of 10 kg milk is predicted to lose 1.4 (95% CI: 0.0–2.9) kg/d if left untreated (CM). In case of QDO treatment, the predicted production loss is 5.7 (95% CI: 4.1–7.4) kg/d, corresponding to an increased loss of 4.3 kg/d. If the cow instead presents on 175 d after calving with a Q1 yield of 6.5 kg/d, the expected production loss if she remains untreated is 2.0 (95% CI: 1.3–2.6) kg/d, whereas the production loss at QDO is 6.3 (95% CI: 5.6–7.0) kg/d. This constitutes an increased production loss of 4.3 kg/d at QDO treatment, which equals that obtained at 70 DIM despite the reduction in prior Q1 yield of 3.5 kg/d.

When the 2 different QDO procedures, abrupt QDO and gradual QDO, were introduced into the multivariable model the production loss was generally predicted to be lower at abrupt QDO than at gradual QDO. However, the difference in production loss between the

2 procedures was not significant. To illustrate, a multiparous cow 175 DIM was predicted have a production loss of 6.0 (95% CI: 4.7–7.3) kg/d at abrupt QDO and a production loss of 6.4 (95% CI: 5.6–7.1) at gradual QDO. A significant difference in production loss was not observed when the variables of precipitates in milk, quarter swelling, and signs of pain at d 0 were introduced into the multivariable model.

DISCUSSION

Several cows exhibited clinical signs associated with mastitis at treatment start, as the farmers observed precipitates in milk (19%), quarter swelling (11%), and signs of pain (1%) at this time point. Thus, although elevated SCC instituted the inclusion criteria, the inflammation in a proportion of the cows did not classify as subclinical. Additionally, one cow showed signs of pain before treatment, indicating a more severe clinical effect.

A proportion of cows exhibited quarter atrophy at treatment start. This might relate to lactation stage (Wilde et al., 1999), but might otherwise result from quarter degeneration associated with the inflammation on the quarter (Shuster et al., 1991). The latter possibly indicates a later stage in infection. The observed increase in atrophic quarters in both treatment groups toward the final examination, likewise presumably resulted of later lactation or infection stage or both. However, the significant difference between the treatments at d 40 indicates that some of the observed increase for QDO related to the treatment. This supports the expectation that QDO treatment is associated with an increased quarter degeneration.

At d 7 to 14 from treatment start, QDO was significantly associated with quarter swelling, presumably in most cases a result of distension caused by the accumulating milk in the udder quarter. The significant association with milk leakage observed for the QDO cows in the week following treatment start supported

Table 4. Multivariable analysis on production loss (kg/d) for cows with elevated SCC subjected to either continued milking (CM) or individual quarter dry-off (QDO)

Variable ¹	Level	Estimate	SE	95% CI	P-value
Treatment	CM	Referent			
	QDO	–7.0	2.4	–12; –2.1	
Parity	1	Referent			
	≥2	4.7	1.8	1.0; 8.3	
DIM (cont.)		–0.05	0.013	–0.07; –0.02	
Prior yield on Q1 (kg/d; cont.)		–0.44	0.34	–1.1; 0.24	
Treatment × DIM		0.03	0.007	0.002; 0.047	<0.001
Treatment × Prior yield on Q1		0.91	0.23	0.45; 1.4	<0.001
Parity × Prior yield on Q1		–0.57	0.26	–1.1; –0.04	0.03
DIM × Prior yield on Q1		0.004	0.002	0.0006; 0.0077	0.02

¹Q1 = quarter subjected to treatment. cont. = continuous variable. Terms with × refer to interaction terms.

Figure 1. Predicted production loss for primiparous and multiparous cows with elevated SCC subjected to either continued milking (CM) or quarter dry-off (QDO).

this. The following decrease in prevalence of quarter swelling indicates that within d 40 from start of QDO the milk production on the quarter was reduced to an extent where distention of the quarter ceased. This is in agreement with the quarter degeneration visualized by increased quarter atrophy.

The alterations in the gland tissue were also apparent from registrations on quarter firmness. The evaluated quarter firmness included both firmness related to fluid tension and firmness related to tissue density. The continual relatively high prevalence of increased quarter firmness at d 40 presumably related to increased tissue density associated with quarter atrophy. Meanwhile, the observed significant treatment difference at d 10 related to fluid tension of the quarters subjected to QDO.

Despite the observed quarter degeneration, 35% of the dried-off quarters did not display signs of atrophy at d 40 from treatment start and milk could be obtained from the vast majority of the nonmilking quarters. Only 2 quarters was categorized as dry, indicating that the duration of quarter degeneration related to QDO extended over months. This is in agreement with previous findings suggesting that re-initiation of milking of the mammary quarter 4 wk or more following dry-off was possible (Capuco and Akers, 1999). The long duration before a stable physiological condition of the dried-off quarter is reached is presumably related to the high production levels of the cows.

Overall, the QDO treatment was associated with quarter alterations corresponding to those observed for abruptly ceased milking at initiation of the dry period (Bertulat et al., 2013). The majority of cows experienced some quarter swelling and increased quarter firmness around d 10 from treatment start. The implication of these manifestations is uncertain but may be associated with discomfort for the cows as suggested for the corresponding manifestations at end lactation drying-off in previous studies (Bertulat et al., 2013; Rajala-Schultz et al., 2018). These studies also suggest that milk yield before dry-off is of significance, as higher prior yield was associated with increased udder pressure, increased level of the stress-indicative metabolites, and decreased lying time following dry-off (Bertulat et al., 2013; Rajala-Schultz et al., 2018). The milk yield before QDO may likewise explain some of the observed variation in the clinical outcomes for the present study, but this was not evaluated.

Some QDO cows experienced clinical signs of severe implications, such as fever (13%) and pain (29%). Additionally, some QDO cows exhibited signs of pain related to Q3. Signs of pain related to Q1 was significantly associated with QDO treatment, whereas fever and signs of pain related to Q3 were not. However, a major limitation of the current study is the reduced power associ-

ated with a restricted sample size. Concern regarding the higher prevalence of fever and signs of pain related to Q3 among QDO cows is thus not dismissible. The result on signs of pain related to the adjacent quarters not subjected to treatment raises suspicion that the QDO treatment may increase the cow's susceptibility to clinical manifestation of other infections.

Blinding of the observers was not feasible with the applied study design. This introduces the risk that more attention was provided for observations on the cows subjected to QDO. Thus, some of the differences with respect to clinical outcomes between the treatments may partly reflect an observation bias.

Adverse implications of the QDO was not evident from changes in behavior during milking as assessed in the current study. The observed levels for frequency of tripping and kicking during milking were comparable to those obtained by Fogsgaard et al. (2015a) for control cows representing cows with SCC <100,000 cells/mL milked in automatic milking units. It was hypothesized that an increase in frequency of tripping or kicking might be observed for cows subjected to QDO due to discomfort related to the treatment. This was not apparent from the obtained data. However, the limited power impairs further conclusion on this. Changes in behavior during milking could also have been evaluated through milking intervals, robot visit frequencies, or incomplete milking events. This was not done in the present study, but may advantageously be done in future studies.

The observed decrease in production loss for both primiparous and multiparous CM cows with increasing DIM possible reflected that an udder inflammation presenting earlier in lactation had a more severe negative effect on production. This effect of DIM was more pronounced at lower prior yield on the quarter identified with the highest CMT score (Q1). Low quarter yield may result either from a late lactation stage or from the inflammation affecting the quarter (Shuster et al., 1991; Wilde et al., 1999). If an early lactation cow presents with a low quarter yield on a quarter with elevated SCC, presumably the inflammation has greater cause in the low yield. Speculatively, the inflammation is more severe and may thus be associated with greater production losses than that observed for the late lactation cow.

The general observation that primiparous cows experienced lower production losses than multiparous cows is in agreement with previous studies demonstrating a greater production loss for multiparous than primiparous cows at clinical and subclinical mastitis (Halasa et al., 2009; Fogsgaard et al., 2015b). Additionally, it may partly reflect a greater lactation persistency for primiparous cows (Friggens et al., 1999). However, at

higher Q1 yield the production losses for primiparous cows exceeded that of multiparous cows, resulting from a greater general dependence on prior Q1 yield for the primiparous cows compared with multiparous cows.

The observed increase in production loss with increasing prior Q1 yield for QDO cows agrees with the expectation that ceased milking of a quarter with a higher yield results in a higher production loss. The increase in production loss with 1 kg/d increase in prior Q1 yield was below 1 kg/d for the majority of cows, indicating that the quarters remaining in production were able to compensate for some of the production loss at ceased quarter milking. Noteworthy, early lactation cows had a greater compensatory production potential at QDO than late lactation cows, suggesting that the gland tissue is more prone to adaptation shortly after calving. However, the compensatory potential of primiparous cows was limited compared with older cows. These observations are in agreement with a previous study demonstrating a compensatory yield increase from milked quarters at dry-off of one or more adjacent quarters (Hamann and Reichmuth, 1990). The study likewise detected a reduced compensatory potential for late lactation primiparous cows.

As a result of the interplay between treatment, prior Q1 yield, and DIM, early lactation cows with high Q1 yield experienced production losses comparable to those of cows later in lactation with lower Q1 yield. This was especially apparent for multiparous cows. Consequently, initiating QDO on cows with relatively high Q1 yield early in lactation is not a major concern with regard to the effect on production loss, while enabling the potential advantage of reduced transmission to other cows to take effect earlier. When considering the potential advantage of reduced transmission, it is, however, important to note the increased risk of milk leakage associated with the QDO in the week following treatment start. The milk leakage may attribute to increased transmission by increased contamination of milking equipment, bedding materials, and so on, by the infected milk.

Two outliers exhibited high production losses compared with the remaining cows. The CM cow was treated for mastitis with antibiotics 3 d following study treatment start and the observed magnitude in production loss was presumably related to the severity of infection. The QDO cow was identified with signs of pain at d 7, thus also indicating a relatively high severity of infection. However, the underlying explanation for the greater production loss remains uncertain.

Herd was included as a fixed variable in the multivariable analysis on production loss due to the expectation that the limited number of study herds did not accommodate the assumption that the herds could be

considered a random sample from the population of organic herds. As herd was eliminated at model reduction, the reported results were not corrected for variation between herds and independence of cow observations within herds. However, at investigation of herd as a random effect the obtained interclass correlation of 0.002 suggested that herd accounted for less than 0.5% of the total variation in the data. Thus, implications on the reported results on production loss is limited.

As expected, the most frequently isolated pathogens were those often associated with subclinical mastitis. The proportion of samples showing no growth was seemingly reasonable when considering the current inclusion threshold of 400,000 cells/mL. It was slightly higher than those generally reported for samples of clinical mastitis but lower than several reports for subclinical mastitis (Taponen et al., 2009). A previous study demonstrated that production loss associated with mastitis is species dependent (Grohn et al., 2004). Furthermore, time since infection influences the production loss (Hertl et al., 2014). Pathogenic species and time since infection may account for some of the variability observed in the current study, but these aspects remain unassessed. The study design resulted in a delay between detection of elevated SCC at milk recording and treatment start ranging from 5 to 36 d. Additionally, the deployed inclusion criterion of an increase in SCC from below to above 400,000 cells/mL does not have high specificity for identification of new infections, as SCC may vary considerably for chronic infections. Considering the aim of reduced transmission, initiation of QDO treatment shortly after infection is advantageous. However, the QDO treatment is less relevant for transient infections with high spontaneous cure rate and short duration of bacterial shedding. This suggests that an inclusion criterion aimed at early identification of chronically infected cows might be more relevant.

Two different procedures were used for QDO: abrupt cessation of milking or gradual cessation of milking. Gradual QDO has greater resemblance to the natural drying at weaning of the offspring. Thus, gradual QDO could be expected to ease the physiological transition of the quarter tissue. However, no significant difference between the 2 procedures with respect to clinical outcomes or production loss was observed. This suggests that there is no advantage of gradual dry-off with respect to the investigated outcomes. Again, the limited power of the study could mean that potential differences between the procedures are not big enough to be detected as significant. However, because the prevalence of swelling, increased firmness, and signs of pain was numerically greater among quarters subjected to gradual QDO and the production loss was likewise greater, an improved power of the study would not

imply any advantage of gradual QDO on these points. A further complication of interpreting the results is the confounding between QDO procedures and herd because only one procedure was practiced within each herd. Furthermore, prior milk yield may be confounding on the results not corrected for this.

The QDO procedures were not suitable for all studied cases because the implications in some cases were comprehensive production loss and development of fever or signs of pain. However, the majority of cows seemingly experienced only mild effects of treatment. Further assessment of the welfare-related implications is necessary to confirm this. Additional studies may also enable identification of characteristics of subjects for which QDO treatment may be beneficial. An important prerequisite for applicability of QDO treatment is that the production loss is matched by reduced transmission to other cows. It is noteworthy that the inclusion criteria applied in the study were quite restrictive as only 70 cows were included during the course of the study period. Particularly, the CMT criterion excluded a proportion of cows. External validity of the results does not extend beyond the employed inclusion criteria.

CONCLUSIONS

The QDO treatment was significantly associated with quarter swelling and increased quarter firmness around d 10 following treatment start. Furthermore, QDO was significantly associated with quarter atrophy around d 40 from treatment start. Hereby, the clinical implications of QDO treatment resembled those observed at abrupt cessation of milking at initiation of the dry period. The gland degeneration in response to QDO extended over months, as 35% of the dried-off quarters remained nonatrophic around d 40 from treatment start and only 6% of the quarters were completely dry. The QDO was significantly associated with signs of pain related to the dried-off quarter, and the treated cows exhibited a nonsignificant but higher prevalence of fever and signs of pain related to the nontreated quarters when compared with negative controls. This indicates that some cows experienced severely negative clinical effects of QDO. The effect on behavior during milking of the treatment remains uncertain due to a low sample size, but no obvious effects were observed. On average the production loss associated with QDO was 4.1 kg/d (95% CI: 3.1–5.0) greater than that observed for the negative controls. However, the production loss depended on parity, DIM and prior yield on the quarter subjected to dry-off (Q1). An increase in production loss with increasing prior yield on Q1 was observed. This increase was below 1 for the majority of cows demonstrating a compensatory production potential of

the nontreated quarters. Early lactation multiparous cows showed the greatest compensatory potential. Our results indicate that individual QDO in management of subclinical mastitis might be feasible for a selected proportion of cows. However, selection criteria need to be further investigated.

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