

Research Article

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Udder health of dairy cows with an extended voluntary waiting period from calving until the first insemination

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Abstract

This study aimed to evaluate the effect of an extended voluntary waiting period (VWP) on SCC, SCC elevations and clinical mastitis incidence during the complete lactation and the first 6 weeks of the next lactation. Holstein-Friesian dairy cows ($N = 154$) were blocked for parity, expected milk yield, calving season and breeding value for persistency and were randomly distributed across 3 VWP (50, 125, or 200 d: VWP-50, VWP-125, VWP-200). Cows were monitored from calving until 6 weeks into the next lactation, or until culling. An elevation of SCC in milk was defined as SCC in milk $\geq 200\,000$ cells/ml after two previous weeks with SCC $< 200\,000$ cells/ml. Over the complete lactation, extending the VWP did not affect SCC elevations and the occurrence of clinical mastitis per lactation or per cow per year. There was no clear effect of VWP length on SCC in the complete lactation, except that multiparous cows in VWP-125 had a higher SCC compared with multiparous cows in VWP-50. Dry-off antibiotic usage per cow per year was lower in VWP-200 compared with VWP-50 for multiparous cows. In the first 6 weeks of the next lactation, cows in VWP-200 had a higher SCC compared with cows in VWP-50, with no effect of VWP on the number of elevations of SCC or the occurrence of clinical mastitis. Extending the VWP may therefore be used to reduce the frequency of transition periods and the associated use of dry-cow antibiotics, with limited impact on udder health, and a similar occurrence of SCC elevations and clinical mastitis per year.

Considering the greatest economic results, indicated by yearly peak milk, a 1-year calving interval (CI) is generally advised to dairy farmers as the optimum lactation cycle (Hanks and Kossaibati, 2012; Temesgen *et al.*, 2022). This 1-year CI usually includes a 10-month lactation, and a 2-month dry period (Kolver *et al.*, 2006; Auldist *et al.*, 2007). As a consequence of this yearly lactation cycle, cows experience multiple transitions every year, including drying-off, calving and the start of the next lactation. During these transitions, large changes in both physiology (e.g. calving, onset of lactation) and management (e.g. regrouping, start of milking) are associated with an increased risk of diseases and disorders, such as clinical mastitis, hypocalcemia, and ketosis (Butler, 2000; Friggens *et al.*, 2004; Fetrow *et al.*, 2006; Pinedo *et al.*, 2014) and possibly culling (Olechnowicz and Jaskowski, 2011). In total, approximately 75% of disease incidences within herds occur within the first month of lactation (Erb *et al.*, 1985; Ingvarsten *et al.*, 2003; LeBlanc *et al.*, 2006).

One possible solution to reduce the frequency of transitions and associated health disorders in dairy cows is to extend the lactation length and CI. Extending the voluntary waiting period from calving until first insemination (VWP) is one of the strategies to extend the lactation length beyond 305 d (Österman and Bertilsson, 2003; Knight, 2005; Lehmann, 2016; Sehested *et al.*, 2019), resulting in an extended lactation length and CI. With an extended CI, the risk of diseases per year can be expected to reduce as there will be fewer calving events per year (Sehested *et al.*, 2019). Cows with an extended VWP (150 d) had a lower incidence of metabolic disorders, lower veterinary costs, and lower culling rates compared with cows with a short VWP (60 d) (van Amburgh *et al.*, 1997). Moreover, a 1-year CI was also associated with a high proportion of cows that were dried off at high production levels (above 18 kg/d), which probably has a negative impact on udder health (Österman and Bertilsson, 2003). For every 5-kg increase in milk yield at dry-off above 12.5 kg, the odds of a cow having an intramammary infection (IMI) with an environmental pathogen at calving increased by at least 77% (Rajala-Schultz *et al.*, 2005). Extending the VWP from 40 to 180 d resulted in a greater proportion (34.2 v. 54.6%) of cows being dried off at lower milk yields (< 15 kg) (Niozas *et al.*, 2019), which could reduce udder health problems around calving related to high milk production at dry-off (Rehn *et al.*, 2000; Rajala-Schultz *et al.*, 2005; Odensten *et al.*, 2007). The

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incidence rate of 12 different diseases, including mastitis, decreased by 9.9 or 19.7% when all cows were managed for a CI of 15 or 17 months, respectively, in comparison with a CI of 13 months (Lehmann, 2016).

Extending the VWP may also have negative consequences on udder health. Cows with an extended CI have more days in late lactation, and cows in late lactation have a greater risk for increased SCC (Singh and Ludri, 2001) related to the decline in milk yield (Hagnestam-Nielsen *et al.*, 2009) and low tight junction integrity (Nguyen *et al.*, 2001). A lower milk yield could have a lower dilution effect and result in a greater SCC (Steenefeld *et al.*, 2013). The low tight junction integrity prior to parturition is related to an increased number of epithelial cells in milk derived from augmented cell shedding and apoptosis which might also contribute to the higher SCC (Kessler *et al.*, 2019). Niozas *et al.* (2019) reported a gradual increase of SCC with increasing days in milk (DIM), but no difference among 3 VWP groups (40, 120, and 180 d) regarding the SCC and incidence of clinical mastitis up to 330 DIM. To our knowledge, the impact of extending the VWP on SCC or incidence of clinical mastitis during the complete lactation and in the first weeks of the subsequent lactation has not yet been reported. The aim of this study was, therefore, to evaluate the effect of 3 VWP lengths (50, 125, and 200 d) on SCC, SCC elevations and clinical mastitis incidence during the complete lactation and the start of the subsequent lactation.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (Netherlands) and complies with the Dutch law on Animal Experimentation (protocol number 2016.D-0038.005).

Animals, experimental design and housing

The experiment was conducted at the Dairy Campus research farm (Leeuwarden, Netherlands) between December 2017 and January 2020. The experimental design, cow management and diet composition have been reported previously (Burgers *et al.*, 2021b). In summary, Holstein Friesian cows ($N = 154$) were selected based on (1) no twin pregnancy, (2) no clinical mastitis or $SCC > 250\,000$ at the final 2 milk test days before dry-off and (3) expected to finish a complete lactation. In week 6 after calving, cows were blocked for parity, calving season, milk yield in the previous lactation (multiparous cows) or expected milk yield (primiparous cows), and the breeding value for persistency (CRV, Arnhem, Netherlands). The experiment started with 50 blocks, each block consisted of 3 cows. After the removal of 2 cows before the end of VWP due to culling as a result of disease, 2 more blocks of 3 cows were added. The cows were randomly distributed within blocks over 3 treatment groups: a VWP of 50 d (VWP-50), 125 d (VWP-125) or 200 d (VWP-200). Cows were inseminated when estrous was detected after the end of VWP. Estrous detection was visually by the animal caretaker as well as automatically using the Nedap Smarttag system. Cows were inseminated until 300 DIM, in other words, cows in VWP-50 had 250 d to conceive, cows in VWP-125 had 175 d to conceive, and cows in VWP-200 had 100 d to conceive. Cows that did not conceive within 300 DIM stayed in the experiment until 530 DIM as long as they produced at least 10 l of milk/d. Cows were milked twice daily around 6 am and 6 pm in a 40-cow rotary milking parlor (GEA,

Dusseldorf, Germany). The experimental period started at calving and ended 6 weeks after the next calving, or at 530 DIM if cows were not pregnant. Animals that were culled were followed until the moment they left the farm.

The partial mixed ration for lactating cows consisted of grass silage, corn silage, soybean meal and wheat meal, supporting 22 kg of milk. Concentrate supply started at 1 kg per day on the day of calving and increased stepwise to 9 kg (primiparous) or 10 kg (multiparous) per day from day 21 onward. After 100 DIM, individual concentrate supply was decreased to match reductions in milk production based on the last 5 d of milk production. In the milking parlor, 1 kg of additional concentrate was supplied daily. The ration for dry cows consisted of grass silage and corn silage, supplemented with wheat straw and concentrate. In the last 10 d before the expected calving date, cows received 1 kg concentrate daily. Cows were dried off between 42 and 49 d before the expected calving date. From 7 d before dry-off, cows were given the dry-cow ration. From 4 d before dry-off, cows were milked once daily. When cows had an average $SCC > 150\,000$ cells/ml or at least one case of clinical mastitis in the complete lactation, cows were treated with antibiotics at dry-off (Orbenin Dry Cow Extra, Zoetis, Netherlands). All cows were treated with teat sealant at dry-off (Orbeseal, Zoetis, Netherlands).

Clinical mastitis was diagnosed and recorded by the staff at the Dairy Campus research herd, during the morning or evening milking. A case of clinical mastitis was defined as a case of visibly abnormal milk, visible changes in the udder due to inflammation, or both. All cows with clinical mastitis were treated with antibiotics according to the herd-specific treatment plan based on the severity of the disease.

Milk collection and analysis

Milk yield was recorded from the day of calving until dry-off and the first 6 weeks of the next lactation at every milking. Milk samples for SCC analysis (ISO 9622, 2013; Qlip, Zutphen, Netherlands) were collected 4 times per week (Tuesday afternoon, Wednesday morning, Wednesday afternoon and Thursday morning), and were analyzed as a pooled sample of 2-morning milkings and 2-afternoon milkings per cow per week.

Statistical analyses

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The natural logarithm of SCC (cells $\times 10^3$ /ml) was used for statistical analyses to approximate normality. Significance of effects was declared at $P < 0.05$.

SCC, elevations of SCC and clinical mastitis of cows with different VWP lengths

Analyses were done for 3 lactation periods: the complete lactation, the 9 weeks before dry-off and the first 6 weeks of the subsequent lactation. To obtain SCC corrected for milk yield (to account for possible dilution effects), average weekly milk yield (kg/d) was included in the model for SCC (Steenefeld *et al.*, 2013). Per lactation period, weekly milk yield and SCC were analyzed using a mixed linear model (PROC MIXED) with the cow as the repeated subject (Model 1). Fixed effects of treatment (VWP-50, VWP-125, or VWP-200), parity class (1 or ≥ 2), week (only for lactation periods of fixed length, i.e. the 9 weeks before dry-off and the first 6 weeks in subsequent lactation) and their 2-way interactions were included in

the model. A first-order autoregressive covariance matrix was the best fit according to the Akaike information criterion and was used to account for within-cow variation.

An elevation of SCC was defined as $SCC \geq 200\,000$ cells/ml after 2 previous weeks with $SCC < 200\,000$ cells/ml (Schukken *et al.*, 2003). The binary variables 'at least 1 elevation of SCC' and 'at least 1 case of clinical mastitis' in the 3 defined lactation periods were analyzed using a generalized linear regression model with logit link function (PROC GLIMMIX; Model 2). Fixed effects of treatment (VWP-50, VWP-125, or VWP-200), parity class (1 or ≥ 2), and their 2-way interactions were included in the model. The number of elevations of SCC and number of cases of clinical mastitis per cow in the complete lactation and the subsequent lactation after different VWP lengths were analyzed using a Poisson distribution for the dependent variable and the default log link function, with the same fixed factors as model 2 (Model 3). The number of SCC elevations per cow per year was calculated by dividing total elevations per lactation by CI length and subsequently multiplying by 365. For the non-pregnant and culled cows, the number of elevations was divided by lactation length. Effects of VWP treatment and parity class on the occurrences of clinical mastitis per cow per year and dry-off antibiotic use per cow per year were assessed through non-parametric tests. Effects of VWP, parity class and of the 6 parity \times treatment groups were tested separately using Kruskal-Wallis tests (PROC nparway; Model 4). Post-hoc comparisons were made between treatment groups of the same parity using Wilcoxon multiple comparisons.

To evaluate the time to the first elevation of SCC or time to the first case of clinical mastitis after different VWP, a survival analysis was used to obtain Kaplan-Meier curves (PROC LIFETEST; Model 5). To evaluate statistical differences in Kaplan-Meier curves among the different VWP treatments, a Cox proportional hazards model was used (PROC PHREG).

Occurrence of high SCC across the dry period

To evaluate whether the SCC status of cows at the onset of a new lactation was related to SCC status before the end of the previous lactation, the SCC recorded at week 10 or 9 prepartum was compared with the first SCC recorded between 10 and 24 DIM (Van Hoeij *et al.*, 2018). Somatic cell count was considered

high when SCC was $\geq 200\,000$ cells/ml (Schukken *et al.*, 2003). Cows were classified as having a chronically high SCC (SCC $\geq 200\,000$ cells/ml before and after calving), cured high SCC (SCC $\geq 200\,000$ cells/ml before and SCC $< 200\,000$ cells/ml after calving), new high SCC (SCC $< 200\,000$ cells/ml before and SCC $\geq 200\,000$ cells/ml after calving), or consistently low SCC (SCC $< 200\,000$ cells/ml before and after calving). The difference in the incidence of postpartum high SCC (binary; 0 or 1) between different VWP lengths was analyzed within cows with low or high SCC before the DP (binary; 0 or 1) using a logistic regression model (Model 6; PROC LOGISTIC in SAS 9.4; SAS Institute Inc., Cary, NC).

Values are presented as least squares means \pm standard error of the mean. All *P*-values of pair-wise comparisons of least squares means were corrected with a Bonferroni adjustment.

Results

This experiment started with 154 cows in total. Within the experiment, 14 cows did not become pregnant during the first lactation within the experiment (2 from VWP-50, 3 from VWP-125, 9 from VWP-200) and 13 cows were culled due to health issues (5 from VWP-50, 4 from VWP-125, 4 from VWP-200). As a result, 127 cows were followed for a complete lactation and 6 weeks into the second lactation. The mean CI of these 127 cows was 384, 452, and 501 d for cows in VWP-50, VWP-125, and VWP-200 (Table 1), and dry period length did not differ. The lactation length of all 154 cows was 363, 445, or 481 d for cows in VWP-50, VWP-125, or VWP-200.

Udder health in the complete lactation

There was no clear directional effect of VWP length on SCC in the complete lactation, yet there was an interaction effect of VWP class and parity (Table 2). Multiparous cows in VWP-125 had a greater SCC than multiparous cows in VWP-50 (5.02 *v.* 4.74 log-transformed $SCC \times 10^3$ cells/ml, $P = 0.01$; online Supplementary Figure S1). Extending VWP did not have an effect on the occurrence of at least 1 elevation of SCC, the number of elevations of SCC per affected cow or the number of SCC elevations per cow per CI or per year. Extending VWP did not have an effect on the occurrence of at least 1 case of clinical mastitis, the total occurrence of clinical mastitis per treated cow or the

Table 1. Calving interval and dry period length of the 127 cows that had a second calf, and lactation length of all 154 cows within the experiment after a voluntary waiting period from calving until the first insemination of 50, 125, or 200 d (VWP-50, VWP-125, or VWP-200)

	VWP			SEM	P-Value
	VWP50	VWP125	VWP200		
Cows, <i>n</i>	47	42	38		
Calving interval, <i>d</i>	384 ^a	452 ^b	501 ^c	7.56	<0.01
	(324–565)	(400–586)	(469–575)		
Dry period length, <i>d</i>	41	42	43	1.7	0.69
	(18–63)	(8–72)	(8–75)		
Cows, <i>n</i>	54	49	51		
Lactation length, <i>d</i>	363 ^a	445 ^b	481 ^b	12.9	0.02
	(283–528)	(361–543)	(422–526)		

^{a,b,c}Values within a row with different superscript letters differ ($P < 0.05$).

Table 2. Variables regarding udder health for cows that were pregnant with a voluntary waiting period from calving until the first insemination of 50, 125, or 200 d (VWP-50, VWP-125, or VWP-200) in the complete lactation

	VWP				Parity			P-value ^a		
	VWP50	VWP125	VWP200	SEM	1	≥2	SEM	VWP	Par	VWP×Par
Cows, <i>n</i>	47	42	38		36	91				
Average SCC ^b within full lactation	4.32	4.45	4.41	0.07	3.90	4.88	0.07	0.37	<0.01	0.02
Average SCC ^b corrected for milk yield	4.28	4.35	4.28	0.07	3.73	4.88	0.06	0.64	<0.01	0.07
Elevations of SCC (% of cows)	77.8	69.6	81.6	7.13	66.5	86.2	6.34	0.41	<0.01	0.08
Elevations of SCC (<i>n</i> cases/affected cow)	2.0	2.4	2.2	0.32	1.5	2.8	0.30	0.64	<0.01	0.36
Elevations of SCC (<i>n</i> , per lactation)	1.6	1.6	1.9	0.30	1.0	2.4	0.26	0.73	<0.01	0.15
Elevations of SCC (<i>n</i> , per cow per year)	1.4	1.3	1.2	0.26	0.85	0.93	0.16	0.74	<0.01	0.21
Clinical mastitis (% of cows)	17.1	26.8	24.5	8.43	11.0	34.6	7.51	0.62	<0.01	0.93
Clinical mastitis (<i>n</i> cases/affected cow)	1.2	1.7	1.5	0.7	1.3	2.0	1.2	0.70	<0.01	0.75
Clinical mastitis (<i>n</i> , per lactation)	0.2	0.5	0.4	0.2	0.1	0.7	0.2	0.44	<0.01	0.78

^aVWP = voluntary waiting period; Par = parity class (parity = 1 and parity ≥2); VWP × Par = interaction of VWP with parity.

^bSomatic cell count ($\times 10^3$ cells/ml) is shown and analyzed as the natural logarithm of SCC.

occurrence of clinical mastitis per lactation, or per year (Table 2). Survival time before elevation of SCC and the development of clinical mastitis was not different among cows with different length of VWP (online Supplementary Figure S2).

Udder health before dry-off

The VWP did not affect the percentage of cows that were dried off with antibiotics (Table 3). However, due to the longer lactation length and CI, the dry-cow antibiotic use per cow per year for multiparous cows was lower in VWP-200 than in VWP-50 (0.37 *v.* 0.65, $P < 0.01$; online Supplementary Table S2). During the 9 weeks before dry-off, the occurrence of at least 1 elevation

of SCC and the number of elevations of SCC did not differ among VWP treatments. There was no clinical mastitis diagnosed in the 9 weeks before dry-off (Table 3).

The milk yield of cows in VWP-50 was higher compared with cows in VWP-125 and VWP-200, whereas there was no effect of VWP on average SCC. Over the 9 weeks before dry-off, SCC increased and milk yield decreased (Fig. 1a, b).

Occurrence of high SCC across the dry period after different VWP lengths

Based on weekly milk samples, the occurrence of SCC $\geq 200\,000$ cells/ml on the last test day before dry-off and the first test day

Table 3. Variables regarding udder health and milk yield for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 d (VWP-50, VWP-125, or VWP-200) in the 9 weeks before dry-off

	VWP				Parity			P-value ¹		
	VWP50	VWP125	VWP200	SEM	1	≥2	SEM	VWP	Par	VWP × Par
Cows with dry period ² , <i>n</i>	47	42	38		36	91				
Dry-off antibiotic use (% of cows)	48.1	28.1	30.4	0.40	20.6	33.4	1.5	0.17	<0.01	0.24
Week -9 until -1 relative to dry-off										
Elevations of SCC ³ (% of cows)	17.8	7.2	16.7	9.0	14.0	27.0	7.0	0.33	<0.01	0.33
Elevations of SCC (<i>n</i> cases/affected cows)	1.2	1.1	1.2	0.07	1.1	1.3	0.1	0.33	<0.01	0.33
Clinical mastitis (% of cows)	0	0	0		0	0				
Milk yield, kg/d ⁴	19.9 ^a	17.2 ^b	16.4 ^b	0.98	19.3	16.4	0.87	0.01	<0.01	0.19
SCC ⁵	4.65	4.56	4.52	0.12	4.03	5.13	0.10	0.63	<0.01	0.32

^{a,b}Values within VWP within a row with different superscript letters differ ($P < 0.05$).

¹VWP = voluntary waiting period; Par = parity class (parity = 1 and parity ≥2); VWP × Par = interaction of VWP with parity.

²Of the 127 cows, 2 cows had a second calving without a dry period.

³Somatic cell count.

⁴ P values for Week, VWP × Week, and Par × Week were all <0.01 for milk yield.

⁵Somatic cell count ($\times 10^3$ cells/ml) is shown and analyzed as the natural logarithm of SCC. P values for week, VWP × Week, and Par × Week were <0.01, 0.40, and 0.85, respectively.

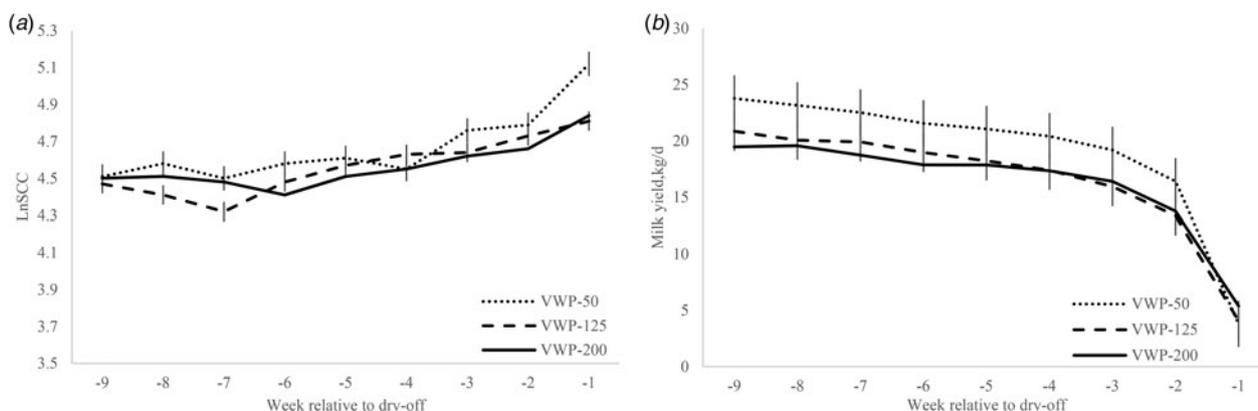


Fig. 1. (a) and (b) Development of somatic cell count (SCC; expressed as the natural logarithm of SCC) (a) and milk yield (b) in cows with a 50 d, 125 d, and 200 d voluntary waiting period (VWP-50, VWP-125, and VWP-200, respectively) during the 9 weeks relative to dry-off at the end of the first lactation. Values represent LSMEANS ± SEM.

after 10 DIM did not differ among VWP treatments (Table 4). Similarly, the proportion of cows with a chronically or cured high SCC or new or no high SCC did not differ among VWP treatments.

Udder health in the subsequent lactation

In the first 6 weeks of the subsequent lactation in the experiment, milk yield was numerically but non-significantly higher for cows in VWP-50 than for cows in VWP-200 ($P = 0.08$; Table 5). The average SCC was greater for cows in VWP-200 than cows in VWP-50 (online Supplementary Figure S3). The VWP did not have an effect on the occurrence of at least 1 elevation of SCC

or at least 1 case of clinical mastitis, the number of elevations of SCC per affected cow, or the number of clinical mastitis cases per treated cow in this period.

Discussion

Extending VWP from calving until the first insemination increased lactation length and CI in our study. Aside from an extended VWP, cows with a short VWP that repeatedly failed to conceive following AI or did not show estrus could also end up with an extended lactation or CI. In this experiment, cows in VWP-50 had a maximum lactation length of 528 d and CI of 565 d. In contrast, cows in VWP-200 had only 100 d to conceive, resulting in a smaller range in CI and fewer cows that became pregnant compared with cows in VWP-50 or VWP-125.

In the current study, extending the VWP did not affect SCC in complete lactation for primiparous cows. For multiparous cows, the SCC for cows with VWP-125 was higher than for cows with VWP-50 (back-transformed median: $367 \text{ v. } 229 \times 10^3 \text{ cells/ml}$), but not for cows with VWP-200. Niozas *et al.* (2019) reported that extending the VWP from 40 d to 120 d or 180 d did not affect SCC throughout the lactation. Österman *et al.* (2005) also found no effects of lactation length on SCC during the complete lactation, when comparing a production system with an 18-month extended CI with a traditional 12-month CI. In addition, in the study of Österman *et al.* (2005), some of the cows were milked 3 times per day to increase their peak milk yield and persistency. Cows with better persistency are more suitable for an extended lactation length (Burgers *et al.*, 2021b). Increasing the frequency of milking had no adverse effect on udder health (Waterman *et al.*, 1983; Wright *et al.*, 2013) or reduced udder diseases (Smith *et al.*, 2002; Dahl *et al.*, 2004; Sitkowska *et al.*, 2018) in different studies. In the current study, no attempt was made to manage the cows for increased persistency.

Extending the VWP did not affect the incidence of SCC elevations and clinical mastitis per lactation or per year. Niozas *et al.* (2019) compared cows with VWP of 40, 120, or 180 d and reported no difference in the number of mastitis cases in 305 d in milk or the whole lactation. However, a lower annual rate of clinical mastitis may be expected when the lactation of cows is extended, due to fewer critical transitions related to dry-off, calving and the start of a new lactation (Allore and Erb, 2000). Such an impact of lactation length on annual disease incidence is only expected if diseases occur more often around calving or in early

Table 4. Mean incidence (%) and number of cows with a low somatic cell count (SCC) (<200 000 cells/ml) or high SCC ($\geq 200\,000$ cells/ml) around the dry period before the second lactation (at the last test-day before dry-off and first test-day after calving) for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 d (VWP-50, VWP-125, or VWP-200)

	VWP			P-value
	VWP50	VWP125	VWP200	
Total cows, n	47	42	38	
	% (n)	% (n)	% (n)	
SCC < 200 000 prepartum	77 (36)	74 (31)	68 (26)	
SCC \geq 200 000 prepartum	23 (11)	26(11)	32 (12)	0.57
SCC < 200 000 postpartum	60 (28)	60 (25)	61 (23)	
SCC \geq 200 000 postpartum	40 (19)	40 (17)	39 (15)	0.86
High SCC prepartum				
Chronic	55 (6)	27 (3)	50 (6)	
Recovered	45 (5)	73 (8)	50 (6)	0.33
Low SCC prepartum				
Elevation	39 (14)	45 (14)	35 (9)	
Healthy	61 (22)	55 (17)	65 (17)	0.64

Table 5. Variables regarding udder health for cows with a voluntary waiting period after calving until the first insemination of 50, 125, or 200 d (VWP-50, VWP-125, or VWP-200) in the first six weeks in the second lactation in the experiment

	VWP			SEM	Parity			<i>P</i> -value ¹		
	VWP50	VWP125	VWP200		2	≥3	SEM	VWP	Par	VWP × Par
Cows, <i>n</i>	47	42	38		36	91				
Milk yield ²	38.3	35.8	34.8	1.20	35.2	37.5	1.06	0.06	0.07	0.31
Average SCC ³	4.25 ^a	4.61 ^{a,b}	4.95 ^b	0.21	4.54	4.67	0.18	0.04	0.54	0.71
Average SCC corrected for milk yield ³	4.31	4.53	4.78	0.16	4.39	4.69	0.17	0.09	0.15	0.40
Elevations of SCC (% of cows)	12.14	23.63	17.31	8.57	19.21	16.18	6.93	0.39	0.66	0.91
Elevations of SCC (<i>n</i> , cases/affected cow)	1.1	1.2	1.2	0.1	1.2	1.2	0.1	0.39	0.66	0.91
Clinical mastitis (% cows)	7.50	4.41	13.46	4.61	5.56	11.36	5.44	0.40	0.29	0.27
Clinical mastitis (<i>n</i> cases/affected cow)	1.1	1.1	1.2	0.0	1.1	1.1	0.1	0.48	0.46	0.32

^{a,b}Values within VWP within a row with different superscript letters differ ($P < 0.05$).

¹VWP = voluntary waiting period; Par = parity class (parity = 1 and parity ≥ 2); VWP × Par = interaction of VWP with parity.

²*P* values for Week, VWP × Week and Par × Week were <0.01, <0.01, and 0.18, respectively for milk yield

³Somatic cell count ($\times 10^3$ cells/ml) is shown and analyzed as the natural logarithm of SCC. *P* values for week, VWP × Week, and Par × Week were <0.01, 0.02, and 0.43, respectively for average SCC; and 0.83, 0.06, and 0.26 *m* respectively for average SCC corrected for milk yield.

lactation. Extending the VWP did not affect the annual rate of mastitis in the current study, which may be explained by the low incidence of clinical mastitis around calving and in early lactation (online Supplementary Figure S4). In the study of Allore and Erb (2000), the annual rate of clinical mastitis was 0.95 and 0.68 cases per cow per year for cows with a standard (50 d) *v.* an extended VWP (150 d), which is much higher than 0.20, 0.23 and 0.19 cases per year for cows in VWP-50, VWP-125, and VWP-200 in the current study.

In the 9 weeks before the dry period, average SCC, SCC elevations, and incidence of clinical mastitis were not affected by an extended VWP. This is in line with Österman *et al.* (2005), who reported that cows with a 15-month CI did not have higher SCC in the last 10 weeks prior to dry-off compared with cows with a 12-month CI. In earlier studies, an increased SCC in late lactation was mainly explained by a lower dilution of the somatic cells in milk due to the lower milk yield in late lactation (Miller *et al.*, 1983), and by the low tight junction integrity before calving, increasing the epithelial cells in milk (Kessler *et al.*, 2019). In the current study, milk yield in the last 9 weeks before dry-off was lower for cows with a 125-d VWP or 200-d VWP, compared with cows with 50-d VWP, while SCC in late lactation was not affected by VWP. Similarly, Pollott (2011) studied cows with different lactation lengths (305 d, 370 d, or 440 d) and reported that throughout lactation, differences in SCC observed between the 3 lactation-length curves were small even in late lactation, with a rise in SCC during pregnancy.

Different VWP had no effect on udder health before dry-off or dry-off antibiotic usage at lactation level. However, due to the longer average CI resulting from extended VWP, annual usage of dry-off antibiotics was lower in VWP-200 compared with VWP-50 for multiparous cows. Our selective use of dry cow antibiotics was based on average SCC and the occurrence of clinical mastitis during lactation. Results indicate that, for multiparous cows, extended VWP may be used to reduce the annual antibiotic use at dry-off. Reduction of annual antibiotics use is of

importance not only as a reduction in veterinary costs but also in relation to the development of bacterial strains which are resistant to antibiotics (Kuipers *et al.*, 2016; Vanhoudt *et al.*, 2018).

Cows with VWP-200 had a higher SCC in the first 6 weeks in the subsequent lactation after the extended VWP and numerically lower milk yield compared with cows with VWP-50 (Burgers *et al.*, 2021b). Partly, the greater postpartum SCC of cows with VWP-200 was explained by the lower milk yield for cows with VWP-200 compared with cows with VWP-50. A lower milk yield could result in a greater SCC because of the lower dilution effect (Steneveld *et al.*, 2013). However, also with a correction for milk yield, SCC in the first 6 weeks of the subsequent lactation was greater for cows with VWP-200, compared with cows with VWP-50. This indicates that the contrast in milk yield did not explain the difference in SCC in the subsequent lactation completely. In addition, fewer cows in VWP-200 were treated with antibiotics at dry-off compared with cows in VWP-50 (30.4% *v.* 48.1% respectively). Not using antibiotics in cows that had a low SCC before dry-off significantly increased the SCC and also incidence rate of clinical mastitis in subsequent lactation (Scherpenzeel *et al.*, 2014). This could also partly explain the higher SCC for cows with VWP-200, compared with cows with VWP-50. In this perspective, the incidence of clinical mastitis during the 6 weeks after the second calving was 13.5% and 7.5% for cows in VWP-200 and VWP-50, respectively, which might also be related to the higher SCC of cows in VWP-200. Multiparous cows with VWP-125 tended to have higher SCC compared with multiparous cows with VWP-50 in the complete lactation. High SCC in VWP-125 was not related to a greater incidence of clinical mastitis, which was not different between VWP treatments with 35.3% and 32.9% for cows with VWP-125 and VWP-50, respectively.

Overall, extending the VWP had limited effects on udder health in the current lactation. In practice, farmers are interested in extending VWP also for other reasons. First, managing cows for extended lactations would lead to fewer transition periods

per cow per year and associated management labor (i.e. drying-off, calving and the start of lactation). Second, fewer calves would be born, and the associated reduction in excess calves and calf care was also a reason for farmers to extend the VWP (Burgers *et al.*, 2021a). Third, a reduction in annual disease occurrence and associated veterinary costs can be expected for diseases specifically associated with the transition period when cows are managed for extended lactations (van Amburgh *et al.*, 1997; Lehmann, 2016).

In conclusion, extending the VWP did not have an effect on the occurrence of SCC elevations and clinical mastitis per lactation or per year. In multiparous cows, cows with VWP-125 had a higher SCC than cows with VWP-50 in complete lactation. Extending the VWP reduced milk yield in the 9 weeks relative to dry-off, but did not affect SCC, or the occurrence of SCC elevations or mastitis in the same period. Dry-off antibiotic usage per year was reduced in VWP-200 compared with VWP-50 for multiparous cows. In the first 6 weeks of the next lactation, SCC was increased after an extended lactation with VWP-200 compared with VWP-50, with no effect on the number of SCC elevations or the occurrence of mastitis. These results indicate that extending the VWP may be used to reduce the frequency of transition periods and the associated use of dry-cow antibiotics with limited impact on udder health.

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