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Invited review

# Dry-off and dairy cow udder health and welfare: Effects of different milk cessation methods



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# ABSTRACT

The cessation of milking at the end of lactation is a routine management practice in dairy herds, and the importance of the dry period for milk production and udder health, has long been recognized. Among countries and herds, drying-off practices differ and include various milk cessation methods, such as changes in milking frequency and in feeding, the use of antibiotic dry cow therapy and teat sealants, and changes in housing. Published studies reporting methods of stopping milk production are scarce, and there are no uniform recommendations on optimal procedures to dry cows off for good udder health, cow welfare, and milk production. This review describes methods to stop mik production to prepare cows for the dry period and their effects on mammary involution, udder health, and dairy cow welfare. Milk yield at dry-off (the final milking at the end of lactation) is important for rapid involution, which stimulates the immune system and promotes good udder health and cow welfare. Based on the findings of this review, gradual cessation of milking over several days before the final milking can effectively reduce milk yield at dry-off and accelerate mammary gland involution while maximizing cow comfort and welfare. Data from this review indicate a target production level of 15 kg/day of milk or less at dry-off.

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### Introduction

In recent decades, dairy production in developed countries has gone through considerable structural changes, with the number of farms declining and herd sizes increasing. Average milk production per cow has increased due to genetic selection and improvements in nutrition and dairy cow management (Barkema et al., 2015), making the transition to the non-lactating state at the end of lactation challenging for high-yielding dairy cows. However, to optimize milk production and udder health in the subsequent lactation, this nonlactating period is extremely important. The beginning of the dry period is a high-risk time in which the cow may acquire new intramammary infections (IMI) (Neave et al., 1950; Oliver and Mitchell, 1983; Smith et al., 1985; Bradley and Green, 2004). Antibiotic dry cow therapy (DCT) has been widely accepted to manage udder health at dry-off and during the dry period, but recent concerns about antimicrobial resistance have increased (OIE, 2016). Animal welfare has also become a major consumer interest (Boogaard et al., 2008; Vanhonacker et al., 2012). Currently, one challenge for the dairy industry is to balance management practices in increasingly intensive production systems with animal health, welfare, and sustainability of production. For dry cow management,

<sup>\*</sup> Corresponding author. E-mail address: paivi.rajala-schultz@helsinki.fi (PJ. Rajala-Schultz). this means optimizing drying-off practices that consider all of these aspects of dairy production.

Various drying-off practices to prepare cows for the dry (nonlactating) period differ greatly among countries, between herds, and sometimes even between cows within herds. Drying-off practices include various milk cessation methods (reduced frequency of milking and changes in feeding), administration of DCT and internal teat sealants, and housing changes. The methods used are either abrupt or gradual dry-off. In the former, all dryingoff practices occur in 1 day, whereas in the latter, they may extend from a few days to several weeks prior to the final milking. Methods of milk cessation vary from milking being stopped on a set day, to milking frequency being reduced over several days or weeks and implemented either with or without restrictions to energy and nutrient intake. Most recently, pharmaceutical interventions also have been studied to aid in this process (Shamay et al., 2003; Boutinaud et al., 2016; Maynou et al., 2018).

The effect of dry-period length on milk production in the subsequent lactation has been broadly investigated (Bachman and Schairer, 2003; Pezeshki et al., 2010; van Knegsel et al., 2013), as has the impact of antibiotic DCT on dairy cow udder health (Eberhart, 1986; Robert et al., 2006; Halasa et al., 2009). Published studies on the actual practices to stop the cow's milk production in preparation for the dry period, however, are surprisingly scarce. No standardized drying-off procedure to optimize udder health, cow welfare, and production in the following lactation exists, likely

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# Table 1

Author, year	Intervention used	Description of methods to stop milk production	Outcome(s) measured (time point)	Notes/factors considered
Wayne and Macy (1933)	<ol> <li>Frequency of milking: only gradual</li> <li>Feeding: no changes</li> </ol>	Half the milk removed from the udder for varying number of days Milkings skipped completely on certain days	SCC	Half udder dried off by different methods Cow-specific protocols
Oliver et al. (1956a)	1) Frequency of milking: stop vs. gradual	1st year: 1x/day for 28 days and then milking once 3, 7, and 14 days later	Milk yield (following lactation)	Selection criteria: cows producing ~4 kg/day of milk or 2 months before calving
	2) Feeding: no changes	2nd year: 1x/day for 14 days, then milking once 3 and 7 days later	IMI (DO, DP, CALV)	caiving
Oliver et al. (1956b)	1) Frequency of milking: stop vs. gradual 2) Feeding: only hay	Stop: Feeding only hay for 48 h before last milking Gradual (cows with ~4 kg/day): 1x/ day for a few weeks	Milk yield (DO, following lactation) IMI (DP, CALV)	
Natzke et al. (1975)	1) Frequency of milking: abrupt vs. gradual	1x/day for 3–4 days	Milk yield (DO)	DCT to 80% of cows
Bushe and Oliver (1987)	2) Feeding: no changes Frequency of milking: abrupt dry-off (group 1) vs. gradual (group 2) vs. gradual with feed changes (group 3)	Group 2: 1x/day for 1 week, skip a day, final milking	IMI (DO, CALV) Milk yield (DO)	No DCT
	(8	Group 3: 1x/day for 1 week, skip a day, final milking + feed restriction: only hay ad libitum	SCC (DRY, DO, DP)	
			Albumin, IgG, lactoferrin (DRY, DO, DP)	
Kelly et al. (1998)	1) Frequency of milking: abrupt vs. gradual	1x/day during d1-d9, d11, d13, d15.	SCC, milk yield (DRY, DO)	Selection criteria: cows with >215 days in milk (DIM)
	2) Feeding changes	From day 9: only silage ration and cut in half	Fat, protein in milk (DRY, DO) PMN, plasmin, albumin,	
Oliver et al. (1990)	1) Frequency of milking: abrupt vs. gradual 2) Feeding changes	Drying-off (7 days), 4 groups: 2x/day + hay only	lactoglobulin (DRY, DO) Milk yield (DRY, DO) IMI (DRY, CALV)	DCT to both quarters of left udder halves of all cows
	2) recuing changes	2x/day + late lactation ration 1x/day + late lactation ration 1x/day + late lactation ration		
Lacy-Hulbert et al. (1999)	1) Frequency of milking: once daily vs. twice daily	Drying-off (21 days), 4 groups:	SCC, milk yield (DO)	Selection criteria: cows between 210 and 245 DIM and with <100,000 cell/mL
	2) Feeding changes	2x/day + 16 kg dry matter (DM)/day	Fat, casein, lactose in milk (DO)	Grazing system
		2x/day + 8 kg DM/day 1x/day + 16 kg DM/day 1x/day + 8 kg DM/day	IgG, BSA (DO)	
Shamay et al. (2003)	1) Frequency of milking: only gradual	2x/day for 3 days	Milk yield (DRY, DO)	Selection criteria: cows with ~300 DIM and <300, 000 cell/mL
	2) Feeding: no changes	CNH (casein hydrolysate) intramammary in two quarters of each cow twice daily for 3 days	Lactose, K, Na, albumin, plasmin, IgG, lactoferrin (DRY, DO, DP)	Thrice daily milking normally
	3) Pharmaceutical intervention		(	
Odensten et al. (2005, 2007)	1) Frequency of milking: only gradual	1x/day, skip 1 day milking, 1x/day	NEFA, urea, glucose in plasma (DRY, DO, DP)	Selection criteria: cows with at least 15 kg of milk/ day and <100,000 cells/mL
	2) Feeding changes	For last 5 days: straw ad libitum vs. 4 kg silage + straw ad libitum	pH, NH3 in rumen fluid (DRY, DP) BCS, heart rate, rectal temperature (DRY, DO) Cortisol, serum amyloid A in plasma (DRY, DO, DP) Milk yield (DO) Fat, protein, lactose in milk (DO)	uay anu ∼100,000 cens/IIIL
Valizaheh et al. (2008)	1) Frequency of milking: only gradual	2x/day for 2 days, 1x/day for 3 days, skip 1 day, 1x/day	SCC (DRY, DO, CALV) IMI (DO, CALV) Feeding and standing behaviour, vocalizations	DCT + ITS to all cows
	50		(DRY, DO)	

Author, year	Intervention used	Description of methods to stop milk production	Outcome(s) measured (time point)	Notes/factors considered
Newman et al. (2009, 2010)	<ol> <li>Frequency of milking: abrupt vs. gradual</li> <li>Feeding: no changes</li> </ol>	1x/day for 4 days, skip 1 day, 1x/day, skip 1 day, 1x/day	Cumulative milk yield for the last week SCC (DRY, DO) Lactoferrin (DRY, DO) IMI (DRY, DO)	DCT to all cows
Tucker et al. (2009)	<ol> <li>Frequency of milking: once vs. twice daily milking</li> </ol>	Drying-off (7 days), 4 groups:	Milk yield (DRY)	Grazing system.
	2) Feeding changes	2x/day + 16 kg DM/day	IMI (DRY, DP)	DCT to all cows after last sample (d15)
		2x/day + 8 kg DM/day 1x/day + 16 kg DM/day	Milk leakage (DRY, DP) Udder characteristics (DRY, DP)	
		1x/day + 8 kg DM/day	Lying times, vocalization (DRY, DP)	
Zobel et al. (2013)	<ol> <li>Frequency of milking: abrupt vs. gradual</li> </ol>	1x/day for 3 days, skip 1 day milking, 1x/day.	Milk yield (DO)	DCT + ITS to all cows
	2) Feeding changes	Ad libitum oat straw + 10 kg of tall fescue grass hay	SCS (DO, CALV)	
			Milk leakage (DP) IMI (CALV) Behaviour, e.g. lying time (DP)	
Ollier et al. (2014)	<ol> <li>Frequency of milking: only abrupt</li> </ol>	Group 1: Ad libitum late lactation diet	Milk yield (DRY)	DCT to all cows
	2) Feeding changes	Group 2: Only hay last 5 days	Prolactin and involution markers, e.g. SCC, BSA, citrate-lactoferrin (DRY, DO, DP)	
	3) Pharmaceutical intervention	Group 3: Late lactation diet + injections of quinagolide IM (d-5 to d+13)		
Gott et al. (2016, 2017)	<ol> <li>Frequency of milking: abrupt vs. gradual</li> </ol>	1x/day final week of lactation	Milk yield (DO, CALV)	DCT to all cows
	2) Feeding: no changes		SCC (DO, CALV) Milk leakage (DO in gradual group, DP) IMI (DO, CALV)	
Rajala-Schultz et al. (2018)	1) Frequency of milking: abrupt vs. gradual	1x/day final week of lactation	Milk yield (DO)	Thrice daily milking normally, DCT to all cows Gott et al. (2016)
	2) Feeding: no changes		Activity and lying behaviour (DP)	
Dancy et al. (2019)	1) Frequency of milking: only gradual	1x/day for 3 days, skip 1day milking, 1x/day	Milk yield, SCC (DRY, DO)	Selection criteria: cows producing $\geq$ 10 kg of mill day
	2) Feeding changes	Ad libitum of same feed ingredients (different proportions): higher nutrient density vs. lower nutrient density	Feeding and lying behaviour, rumination (DRY, DO, DP)	DCT + ITS to all cows
			NEFA, haptoglobin in plasma (DRY, DO, DP) BCS, body weight (DRY, DO, DP)	

DRY, Drying-off; DO, dry-off; DP, dry period; CALV, after calving; SCC, somatic cell counts; IMI, intramammary infections; PMN, polymorphonuclear leukocyte; BSA, bovine serum albumin; NEFA, non-esterified fatty acids; BCS, body condition score; DCT, antibiotic dry cow therapy; ITS, internal teat sealant; DIM, days in milk.

because of the dependence on herd and cow characteristics. Additionally, terminology for drying-off practices can be confusing, with differences across regions and languages. In this review, we use the term 'dry-off' to refer to the final milking at the end of lactation before the dry period, and 'drying-off' to refer to the period of implementation of measures to prepare cows for the dry period; this may last from a few days to several weeks. Finally, 'milk cessation methods' in this review refer to those practices deployed to stop the cow's milk production, including changes in frequency of milking or changes in feeding, or the administration of pharmaceutical products.

This review focuses on milk cessation methods used to prepare cows for the dry period. The aim is to describe reported methods and summarize the effects of these methods on milk yield at dryoff, on mammary involution, on udder health, and on cow welfare.

# Literature search

This is a narrative review of different milk cessation methods and their effects on the udder health and welfare of dairy cows. The following criteria (O'Connor and Sargeant, 2015) were considerations in conducting the literature search and deciding on publications to be included: (1) the population of interest was dairy cows in late lactation; (2) the intervention included practices during drying-off to prepare cows for the dry period; and (3) the outcomes evaluated were measures of udder health and cow welfare, such as somatic cell count (SCC), IMI status, milk yield, milk composition (natural protective factors in milk), and behaviour (lying behaviour and vocalizations) around dry-off. The keywords for the search were 'drying-off' or 'dry-off 'or 'dry\*', 'mammary gland involution', 'milk cessation' or 'cessation of milking' or 'milking frequency', and 'dairy cow' or 'dairy cattle' in different combinations in a search of two databases<sup>1,2</sup>. Both authors were involved in the search and agreed on articles to be included. They also examined reference lists of the most relevant papers and auxiliary data sources such as the Google Scholar search engine<sup>3</sup>. There were no restrictions on publication year.

Briefly, the search criteria identified 706 articles. Study selection took place in two stages: during the first stage, the title and abstract of the manuscripts were screened and in the second stage the full text were screened. A total of 465 articles were excluded because they did not match the aims of the review. Studies investigating the use of antibiotic DCT or internal teat sealants (50 articles) and dry period length (80 articles) were also excluded, as were studies of mammary gland changes at cellular level during involution and the dry period (37 articles). Review manuscripts (such as invited reviews, 20 articles) were not included in the summary of study results. Finally, 54 studies were included.

### Methods of milk cessation to prepare the cow for the dry period

Two methods to stop milking dairy cows at the end of lactation appear in the literature: (1) abrupt cessation of milking or 'stop milking', when milking is suddenly halted on a set day, based on the expected calving date and desired dry period length; and (2) gradual cessation of milking, intermittent milking, or reduced milking frequency, in which milking is gradually reduced over a period of time up to the final milking at dry-off. These methods may or may not be combined with changes in feeding that aim to reduce the amount of energy or nutrient intake.

Current milk cessation methods vary among countries and among herds. Abrupt cessation of milking is common worldwide; it is used on 74% of dairy farms in the United States,<sup>4</sup> 73% of farms in Germany (Bertulat et al., 2015), and 83% of farms in Scotland (Fujiwara et al., 2018). From a herd-management perspective, abrupt dry-off is relatively easy to implement, especially in large herds that calve year-round, as it can be achieved in a day. In large seasonal herds, however, labor demand for such a process can be challenging when potentially hundreds of cows should be dried off within a very short timeframe. Gradual cessation of milking is frequently used in some countries; in Finland, 96% of farmers dry their cows off gradually (Vilar et al., 2018). The Swedish Dairy Association<sup>5</sup> recommends a 5-day gradual cessation protocol for drying off high-producing cows (daily yield > 25 kg at the start of drying-off).

Gradual dry-off is accomplished by reducing milking frequency with or without changes in feeding. For methods of stopping milk production in preparation for the dry period, see Table 1. A oncedaily (1x) milking schedule for approximately a week before dryoff has been common in research settings (Natzke et al., 1975; Kelly et al., 1998; Gott et al., 2016). Alternative protocols include a 5-day protocol with 1x milking/day on days 1, 2, 3, and 5 (Zobel et al., 2013; Dancy et al., 2019). A reduced energy diet has also been investigated in multiple studies e.g. feeding cows only ad libitum hay or straw (Bushe and Oliver, 1987; Odensten et al., 2007b; Ollier et al., 2014), restricting dry matter intake (Tucker et al., 2009), or feeding higher vs. lower nutrient-density diets (Dancy et al., 2019).

In addition, various pharmaceuticals have been studied as potential aids to stop milk production. These include systemic injection, at or near dry-off, of prolactin inhibitors (Ollier et al., 2013, 2014; Boutinaud et al., 2016; Bertulat et al., 2017), or intramammary infusions of casein hydrolysate or chitosan hydrogel (Shamay et al., 2003; Lanctôt et al., 2017). These products, however, are generally not commercially available.

## Mammary gland involution in brief

Involution is a complex process during which the udder transitions from a lactating to a non-lactating state (Hurley, 1989). Briefly, it begins after milk removal from the mammary gland is reduced (Capuco and Akers, 1999; Silanikove, 2014). If milk is not removed within 16 h, secretion of prolactin and synthesis of milk are supressed (Cunningham and Klein, 2007). During lactation, the tight junctions, extracellular structures between adjacent epithelial cells, are closed. During involution, the tight-junction permeability increases (Hurley, 1989), allowing transfer of substances between blood and milk (Stelwagen et al., 1994). Therefore, during involution, concentration in milk of blood-derived factors such as leukocytes and immunoglobulins increase (Oliver and Sordillo, 1989). At the same time, synthesis of mammary-derived components (such as lactose and casein) is reduced, leading to changes in the composition of mammary secretion during the early dry period (Sordillo and Nickerson, 1988; Hurley, 1989; Oliver and Sordillo, 1989). During involution, non-specific antibacterial substances or natural protective factors in mammary secretions, including lactoferrin, complement, and lysozyme, also increase (Sordillo et al., 1997), making the mammary environment unfavourable for bacterial growth. These antibacterial components are part of the innate immune response, which is the predominant defence in the udder during early stages of infection (Sordillo and Streicher, 2002).

Factors such as pregnancy status and systemic and local regulatory hormones (Capuco and Akers, 1999), as well as dryingoff practices, influence the mammary gland involution process (Stefanon et al., 2002). Concentrations of natural protective factors in the mammary gland increase differently during involution, depending on milk yield at the moment of dry-off. According to Silanikove et al. (2013), metabolic and immunological changes during mammary involution are faster in cows with low milk yield at dry-off than in cows with high milk yield at dryoff. For example, cows with milk yield <14 L/day have higher concentrations of lactoferrin, immunoglobulins, and SCC than those yielding 25–35 L/day at dry-off (Silanikove et al., 2013). These changes allow for more efficient elimination of existing infections and the prevention of new infections. In most cases, elevated SCC at dry-off in cows with overall good udder health is due to a concentration effect that occurs as milk production decreases (Lacy-Hulbert et al., 1999); one of the first signs of mammary involution is also an increase in SCC during drying-off (Sordillo and Nickerson, 1988).

# Effects of milk cessation methods on mammary gland involution

Drying-off practices, including methods to stop milk production, aim to transition the cow into the dry period, accelerate mammary involution, and improve the cow's ability to eliminate existing IMIs and prevent new infections. Gradual cessation of

<sup>&</sup>lt;sup>1</sup> See: National Center for Biotechnology Information (NCBI) Pubmed database. https://www.ncbi.nlm.nih.gov (Accessed 12 November 2018).

<sup>&</sup>lt;sup>2</sup> See: Web of Science. http://login.webofknowledge.com/error/Error?Error=I-PError&PathInfo=%2F&RouterURL=http%3A%2F%2Fwww.webofknowledge.com% 2F&Domain=.webofknowledge.com&Src=IP&Alias=WOK5 (Accessed 12 November 2018).

<sup>&</sup>lt;sup>3</sup> See: Google Scholar search engine. https://scholar.google.com (Accessed 12 November 2018).

<sup>&</sup>lt;sup>4</sup> See: USDA, 2016. Dairy 2014, Milk Quality, Milking Procedures, and Mastitis in the United States, 2014. USDA-APHIS-VS-CEAH-NAHMS. Fort Collins, CO. USA. #704.0916. https://www.aphis.usda.gov/animal\_health/nahms/dairy/downloads/ dairy14/Dairy14\_dr\_Mastitis.pdf (Accessed 26 September 2019).

<sup>&</sup>lt;sup>5</sup> See: Växa Sverige. https://www.vxa.se/fakta/styrning-och-rutiner/SOP/standardrutin-sinlaggning (Accessed 11 February 2020)

milking during drying-off has been associated with increased tight-junction permeability (Stelwagen et al., 1994), and increased concentration of natural protective factors in the mammary gland (Bushe and Oliver, 1987). Lacy-Hulbert et al. (1999) reported that cows milked once daily during late lactation had increased concentrations of immunoglobulin G in milk at dry-off compared to cows with similar milk yield that were milked twice daily. Comparing two groups of cows with similar milk production prior to drving-off. lactoferrin concentration increased significantly among intermittently milked cows during a 1-week drying-off procedure compared to concentration in abruptly dried-off cows (Newman et al., 2009). Moreover, SCC levels were higher at dry-off in the quarters of intermittently milked cows than in those of cows dried off abruptly (Stelwagen et al., 1994; Zobel et al., 2013), independent of quarter infection status. These studies suggest that reduced milking frequency at the end of lactation accelerates the involution process and leads to increases in natural protective substances in milk, thus enhancing the innate defence system during the high-risk period for new IMI.

The involution process can also be hastened through feedingand ration changes. Reduced energy diets during drying-off have resulted in increased SCC at the final milking. During a drying-off procedure that lasted 5 days, Odensten et al. (2007b) reported higher SCC at dry-off in cows fed a straw-only diet compared to those fed a combination straw and silage diet. In addition, Lacy-Hulbert et al. (1999) reported that feed restriction of cows under pasture conditions at the end of lactation increased milk SCC by the time of dry-off. Bushe and Oliver (1987) reported that combining intermittent milking and feeding of a reduced-energy ration resulted in higher concentrations of immunoglobulin G at dry-off compared to levels in cows dried off abruptly or milked intermittently, but without feeding changes. Bushe and Oliver (1987) and Kelly et al. (1998) also observed that, in contrast to cows dried off without any feeding changes, increase in lactoferrin in mammary secretions was more rapid in cows dried off with intermittent milking and with restricted rations.

At or near dry-off, pharmaceutical interventions such as intramammary infusions of casein hydrolysate or of chitosan hydrogel, or injected prolactin inhibitors, also hasten mammary involution (Shamay et al., 2003; Lanctôt et al., 2017). Caseinderived peptides, which are potent inflammatory mediators, occur naturally in the mammary gland during active involution (Shamay et al., 2003) and disrupt tight-junction permeability (Shamay et al., 2003; Ponchon et al., 2014). Chitosan, a polysaccharide produced from chitin, enhances host defences by improving the functions of polymorphonuclear leukocytes and macrophages (Senel and McClure, 2004). According to Lanctôt et al. (2017), chitosan hydrogel activated the immune system in the mammary gland and accelerated the involution process, as shown by increased lactoferrin concentrations in mammary secretions. Finally, in cows injected with the prolactin inhibitors guinagolide (Ollier et al., 2013, 2014) or cabergoline (Boutinaud et al., 2016), lactoferrin concentrations in the mammary secretions increased faster than in secretions of control, untreated cows. Compared to the process in a control group, a single injection of cabergoline, in conjunction with abrupt dry-off, accelerated the involution process by reducing the secretory activity of mammary epithelial cells and by more rapidly increasing lactoferrin concentration and SCC (Boutinaud et al., 2016).

### Milk cessation methods to reduce milk yield at dry-off

Dairy cows can still produce large quantities of milk at the end of lactation; transition to the non-lactating state can thus be extremely challenging and even painful, especially when dry-off is abrupt. Milk cessation methods should aim to reduce such discomfort and mitigate any detrimental effects on cow health and welfare during transition to the dry period. Based on the literature, reducing milk yield prior to dry-off is key to reaching this objective.

Reduction in milk yield following once-daily milking during the final week of lactation ranges from 20-47% (Bushe and Oliver, 1987; Oliver et al., 1990; Tucker et al., 2009; Gott et al., 2016). During the last week of lactation, milk vield decreased from 23.9 kg/day to 14.3 kg/day in cows milked once daily compared to 24.7 kg/day to 22.7 kg in abruptly dried-off cows (Rajala-Schultz et al., 2018). Other studies have also described significantly lower milk yields at dry-off after reducing milking frequencies compared to abrupt dry-off. When cows were milked once daily for 3 days, and then milking was skipped 1 day before the final milking, milk yield dropped from 23.8 kg/day before the intervention to 10.9 kg at dry-off (Zobel et al., 2013). Following a similar 5-day intermittent-milking protocol, Dancy et al. (2019) reported an average decrease of 10 kg/day in milk yield over the drying-off period. Newman et al. (2009) reported that cumulative milk yield in the last week of lactation was significantly lower in gradually dried-off cows than in those that were abruptly dried-off (78.7 kg vs. 125.8 kg). In conclusion, reduced milking frequency at the end of lactation can lower milk vield substantially at dry-off.

Because maintenance of milk production by dairy cows is strongly dependent on the provision of nutrients to the udder (Agenäs et al., 2003), limiting energy and nutrient intake in late lactation can also effectively reduce milk synthesis and volume (Lacy-Hulbert et al., 1999). Strict feed restriction, however, may compromise cow metabolism (Odensten et al., 2007b) and induce hunger (Valizaheh et al., 2008; Tucker et al., 2009). However, as shown by Dancy et al. (2019), diets of lower nutrient density do not necessarily or substantially alter cow physiology (as assessed by rumination activity, reticulorumen pH, blood glucose, haptoglobin, non-esterified fatty acids, and body condition). Recently, acidogenic mineral boluses as feed supplements have been used to reduce dry matter intake, and consequently milk production, prior to dry-off. Such boluses contain anionic salts and induce a temporary metabolic acidosis. In an experimental field trial, the administration of two boluses into the rumen 5 days before the scheduled dry-off reduced milk production by approximately 2 kg on the second day after bolus application; when administered at dry-off, feed intake decreased during the following 3 days (Maynou et al., 2018). A similar effect could also be achieved simply by changing diets. Whereas reducing milking frequency or restricting energy intake alone will reduce milk production, a combination of intermittent milking and reduced-energy ration during the final week of lactation results in the greatest reduction in milk yield before dry-off (Bushe and Oliver, 1987; Oliver et al., 1990). To reduce milk production, feed restriction and ration changes to limit energy/nutrient intake are effective, but they should be implemented carefully to avoid metabolic disturbances or hunger.

Milk production can also be manipulated and reduced with the aid of pharmaceutical products. The maintenance of milk synthesis depends on prolactin secretion, which is dependent on milk removal from the mammary gland. The pharmaceutical products quinagolide and cabergoline inhibit prolactin secretions in the pituitary gland, and their potential role in drying-off has been investigated. Because of their differing half-lives in dairy cows, to achieve similar reductions in prolactin concentrations and to accelerate the involution process requires individualized dosing regimens (Ollier et al., 2013, 2014; Boutinaud et al., 2016). The same effect, reduced milk production, can also be the result of reducing milking frequency, or changing rations, or both.

### Effects of milk cessation methods on udder health

One of the first studies to report the effect of different drying-off methods on udder health took place in the 1930s. Wayne and Macy (1933) studied a limited sample of cows with much lower milk production than currently, but this demonstrates interest in this topic almost 100 years ago. In the 1950s, Oliver et al. (1956a, 1956b) observed that guarters of abruptly dried-off cows were more likely to become infected in the dry period than quarters of cows milked intermittently before the final milking. They also reported that the new dry-period infection rate increased along with higher milk yield at drying-off. Twenty years later, Natzke et al. (1975) reported that guarters of abruptly dried-off cows developed more new infections than quarters of gradually dried-off cows, whether or not those quarters were treated with DCT. These early studies suggested that cows dried off abruptly were more susceptible to new IMI than cows dried off by intermittent milking. It is also noteworthy that milk yields at the time were substantially lower than they are now, further highlighting the importance of reducing milk production prior to dry-off.

Because most IMIs result from bacteria gaining entry to the udder through the teat canal, one of the most important natural defence mechanisms against new IMI after dry-off is the formation of a keratin plug in each teat canal. This plug physically prevents the entry of bacteria into the teat cistern and into the mammary gland. Information as to the length of time needed for keratin plug formation after dry-off, however, is sparse. Dingwell et al. (2004) observed that the median time for teat-canal closure ranged from 1-2 weeks, and also that cows producing >21 kg/day of milk at dryoff had a lower chance of teat closure compared to that of lowerproducing cows. Comalli et al. (1984) reported that teat-canal closure occurs approximately 16 days after cessation of milking. Furthermore, Odensten et al. (2007a) observed more open teat canals at 2-3 weeks after dry-off in high-yielding cows than in low-yielding cows (milk production 17.8-29.5 kg/day vs. 5.0-11.4 kg/day during the last week prior to dry-off, respectively). Similarly, Summers et al. (2004) observed that grazing cows under restricted dry matter intake after the final milking experienced increased teat-canal closure at 7 and 14 days after cessation of milking compared to closure in cows without feed restriction. During the early dry period, when milk is still being synthesized, accumulating milk in the cistern raises internal pressure in the mammary gland (Nickerson, 1989; Bertulat et al., 2013), and this may lead to milk leakage and a delay in the keratinplug formation.

It is therefore not surprising that several studies have reported that the risk of milk leakage is higher in cows with high milk production at dry-off than in those with low production (Bertulat et al., 2013; Silanikove et al., 2013; Gott et al., 2016; Hop et al., 2019). Gott et al. (2016) demonstrated that, above 18 kg, every 4.5kg increase in milk yield at dry-off raises the odds of milk leakage by 2.1-fold. Even though abruptly dried-off cows produced significantly more at dry-off than gradually dried-off cows in that same study, there were no significant differences in the proportion of quarters or cows leaking after drv-off between abrupt and gradual milk cessation: thus milk vield level appeared to be the main driver for milk leakage. Zobel et al. (2013) reported that the probability of leaking milk was lower in cows dried off gradually than abruptly, and that intermittently milked cows produced less at dry-off. An association between restricted dry matter intake (8 kg vs. 16 kg of DM/day during a drying-off period of 1 week) and lower probability of milk leakage has also emerged (Tucker et al., 2009), which could be associated with faster teat-canal closure (Summers et al., 2004). Reduced incidence of milk leakage has also been associated with administration of pharmaceutical products such as cabergoline (Hop et al., 2019). In short, cows with a higher milk yield at dry-off are at greater risk of open teat canals and leaking milk than their lower-producing herdmates. Drying-off practices that reduce milk yield prior to dry-off also promote faster teat-canal closure and keratin-plug formation.

The physical location of the cows immediately after dry-off may also affect milk leakage; if cows in the dry pen can hear the milking machine, they anticipate being milked and may experience milk let down and milk leakage (Stefanowska et al., 2000; Zobel et al., 2013). Literature on this, however, is limited.

The time immediately after dry-off is one of the high-risk periods for acquiring new IMI (Neave et al., 1950; Oliver and Mitchell, 1983; Smith et al., 1985; Bradley and Green, 2004). At that point, the flushing effect of milking has ceased, the concentrations of natural protective factors in milk are low, milk is still synthetized and accumulates in the udder, and the teat canals are not yet closed. Dingwell et al. (2004) reported that open teat canals within the first 3 weeks of the dry period raised the odds of new IMI. Moreover, cows that leaked milk after dry-off had a four-fold greater risk of clinical mastitis in the dry period (Schukken et al., 1993).

Higher milk yield at dry-off elevates the risk of IMI during the dry period and after calving. Newman et al. (2010) reported that high-milk-yield cows with healthy udders at dry-off had significantly higher odds of IMI at calving than healthy cows with low milk yields ( $\geq$  115 kg vs. < 75 kg cumulative milk yield during the final week of lactation). Similarly, every 5 kg increase in milk yield at dry-off above 12.5 kg raised the odds of IMI at calving by 77% (Rajala-Schultz et al., 2005). Odensten et al. (2007a) observed, 1

#### Table 2

Milk production levels at dry-off and their effect on udder health and cow welfare.

Reference	Milk yield threshold for grouping cows	Effect of increasing milk yield at dry-off or effect of high vs. low milk yield at dry-off
Dingwell et al. (2004)	low: < 21 kg/day high: $\geq$ 21 kg/day	Odds of open teat canal 1.8x higher
Rajala-Schultz et al. (2005)	12.5 kg, with 5 kg increase	Odds of IMI at calving 1.8x higher
Odensten et al. (2007a)	low: 5–11.4 kg/day	Higher plasma cortisol levels
	high: 17.8–29.5 kg/day	Higher percentage of open teat canal
		Higher percentage of IMI at calving
Newman et al. (2010)	low: < 10.7 kg/day	Odds of IMI at calving 7x higher
	high: $\geq$ 16.4 kg/day	
Bertulat et al. (2013)	low: < 15 kg/day	Higher udder pressure
	high: > 20 kg/day	Odds of milk leakage 5.1x higher
		Higher increase of faecal glucocorticoid
Silanikove et al. (2013)	low: $\leq$ 14 L/day	Lower lactoferrin concentration
	high: 25–35 L/day	Lower proportion of milk leukocyte population
Gott et al. (2016)	18.1 kg, with 4.5 kg increase	Odds of milk leakage 2x higher
Hop et al. (2019)	low: 13–18 kg/day	Odds of milk leakage 3.2x higher
	high: >18 kg/day	

week after calving, a smaller proportion of IMI in cows with low milk yield than in those with medium or high milk yield at dry-off. Although Gott et al. (2016) did not find significant differences in IMI prevalence at calving between cows dried off gradually or abruptly, they did observe, in the period from dry-off to calving, that there was a smaller proportion of cows with infected quarters in the gradual (16.8%) than in the abrupt cessation group (12.2%)(Gott et al., 2017). This was despite the fact that all cows in both groups received DCT and produced similar amounts of milk on the final test day before intervention (22.2 kg in the gradual group vs. 21.8 kg in the abrupt group). Fewer IMIs in the gradually dried-off cows may be attributable to accelerated mammary involution and higher concentrations of natural protective factors. In summary, methods that reduce milk production, hasten involution, and rapidly elevate natural protective factors in the udder, play an important role in the prevention and control of new IMI during the early dry period (Oliver and Sordillo, 1989).

Concerning pharmaceutical products, when compared to no treatment, the use of cabergoline at dry-off reduced the occurrence of new IMI caused by major pathogens across the dry period by 21% (Hop et al., 2019). As to the relationship between milk yield at dry-off and SCC after calving, results have varied. Some authors have reported high milk yield at dry-off associated with increased probability of high SCC in the subsequent lactation (Green et al., 2008; Madouasse et al., 2012; Gott et al., 2017). On the other hand, other studies show milk yield before dry-off as not significantly associated with SCC after calving (Odensten et al., 2007b; Zobel et al., 2013).

None of the studies reviewed above were specifically designed to evaluate the relationship between optimal milk yield level at dry-off, incidence of new IMI during the dry period, and IMI prevalence at calving. As is clear from Table 2, milk yields of 15 kg/ day or less were generally associated with improved udder health compared to higher milk yields; thus a milk yield of 15 kg/day or less is proposed as the target to be achieved by dry-off. Reducing milking frequency or restricting energy and nutrient intake before dry-off, or both, reduces milk yield and accelerates involution, reduces the probability of milk leakage, and favours rapid formation of the keratin plug. Lowered milk yield prior to dryoff therefore aids in the prevention and control of new IMI during the dry period and subsequent lactation.

### Effects of milk cessation methods on cow welfare

Under natural conditions, calves gradually reduce suckling frequency before weaning (Vitale et al., 1986), and intermittent milking during drying-off resembles this natural phenomenon more closely than abrupt cessation of milking. In addition, gradual milk cessation meets the three aspects of welfare: natural living (natural behaviour), affective state (pleasant and unpleasant feelings), and biological functioning (good health status) more closely than abrupt milk cessation (von Keyserlingk et al., 2009). For a detailed review of the welfare aspects of drying-off, see Zobel et al. (2015).

Cortisol and its metabolite concentrations increase as a consequence of pain, discomfort, or any stressful experience (Rutherford, 2002; Odensten et al., 2007a; Bertulat et al., 2013). Bertulat et al. (2013) observed that higher milk yield at the time of dry-off resulted in higher intramammary pressure and increased fecal glucocorticoid metabolites after dry-off. They concluded that high-producing cows experienced some level of discomfort, and potentially also pain, for several days, due to increasing udder pressure after abrupt cessation of milking. Similarly, Odensten et al. (2007a) reported that cows with a medium (11.5–17.7 kg/day) or high (17.8–29.5 kg/day) milk yield during drying-off showed elevated concentrations of plasma cortisol during this period and after dry-off, whereas low-yielding cows (5.0–11.4 kg/day) did not.

They also suggested that high plasma cortisol levels were associated with high intramammary pressure in cows with high milk production at dry-off. Additionally, the daily plasma cortisol concentrations during drying-off were higher in cows fed only straw than in cows fed straw and silage (Odensten et al., 2007b). Based on these findings, it appears that at dry-off, cows with a high milk yield experience more stress than cows with a low milk yield, especially after abrupt cessation of milking. Further, more restrictive diets may cause more stress or discomfort than less restrictive diets, because they induce hunger.

Changes in lying times and lying bouts can serve as signs of discomfort due to udder pressure (Chapinal et al., 2014). Rajala-Schultz et al. (2018) and Zobel et al. (2013) observed that cows with high milk yield during drying-off had shortened lying bouts and shorter daily lying time after dry-off than lower-producing cows. Lying behaviour was not directly associated with milk cessation method, but gradually dried-off cows with lower milk yield at dry-off tended to have longer lying bouts than abruptly dried-off cows during the first days of the dry period (Rajala-Schultz et al., 2018). Tucker et al. (2009) reported that milking cows once rather than twice daily during the last week of lactation had little effect on behaviour before or after the final milking at dry-off. That study, however, was conducted with grazing cows, and milk yields in the pasture system were generally lower. It has also been reported that the lying behaviour of primiparous cows was more affected by milk yield at dry-off than that of multiparous cows (Rajala-Schultz et al., 2018). Correspondingly, Chapinal et al. (2014) observed that primiparous cows, especially high-yielding cows, reduced their lying time by 2 h/ day immediately after abrupt cessation of milking compared to the baseline time averaged from the 2 days before dry-off. However, lying time did not shorten among multiparous cows, suggesting that different drying-off practices might suit different parity groups.

Vocalizations may be behavioural indicators for such issues as hunger, negative feelings, and pain (Watts and Stookey, 2000). Silanikove et al. (2013) suggested that increased vocalization was suggestive of pain from udder engorgement in cows with high milk yield at dry-off after abrupt milk cessation. Tucker et al. (2009) reported that cows on more restrictive diets (8 kg vs. 16 kg /day of dry matter intake) during drying-off vocalized more, which may indicate that these cows were experiencing hunger. Similarly, Valizaheh et al. (2008) reported that the frequency of calls increased during the first days after changing the lactation ration to hay-only during drying-off. Cows fed a more restrictive and less digestible diet (oat hay) vocalized more than cows fed grass hay, probably because of hunger. This study also reported that although the total feeding time did not differ between cows on different drying-off diets, cows on more restrictive diets spent more inactive standing time in pens without exhibiting feeding behaviour. This, combined with increased vocalizations, could indicate poor welfare of cows during drying-off.

Pharmaceutical products have also been evaluated for their ability to improve cow welfare and to alleviate discomfort from increased internal udder pressure after abrupt dry-off. Udder engorgement was reduced in cows treated with cabergoline; after dry-off, treated cows lay down 1.5 h/day longer than control cows (Bach et al., 2015; Bertulat et al., 2017). Intramammary infusion of casein hydrolysate, which reduced intramammary pressure, made high-yielding cows (average 25 kg of milk/day) that were dried off abruptly seem more tranquil, with longer lying-bout durations and fewer steps than untreated cows (Leitner et al., 2007). The administration of acidogenic boluses 8-12 h before the last milking reduced udder pressure during the first 48 h after dry-off, and also raised daily lying time by 85 min on the first day after dryoff (Maynou et al., 2018). From a cow welfare perspective, increased lying time is generally viewed as positive; what is, however, unclear is whether increased lying time in this study

could have simply been a consequence of cows feeling ill due to temporary metabolic acidosis induced by the acidogenic boluses.

# Conclusions

Different methods to stop the cow's milk production to prepare her for the dry period include abrupt or gradual cessation of milking. changes in feeding, and the use of pharmaceutical products. The time immediately after dry-off is a high-risk period for new IMIs: milk still accumulates in the udder, the keratin plug has not yet completely formed, and the natural protective factors in the mammary gland are still low. High milk yield at dry-off is a risk factor for milk leakage and delayed keratin plug formation, and consequently for new IMIs. Especially in high-producing cows, milk accumulation in the mammary gland after dry-off raises internal udder pressure, increasing stress and discomfort. Reduced milking frequency reduces milk yield before dry-off, hastens mammary gland involution, and elevates natural protective factors in the udder, playing an important role in mammary gland defence over the dry period. Lower milk yield at dry-off can also be achieved by reduced energy intake, but feeding changes during drying-off should be carefully considered, because a restrictive diet can cause stress, hunger and potential metabolic disturbances. Pharmaceutical products to reduce milk yield at dry-off are generally not available commercially, but similar effects are achievable through conventional management practices. The review of the existing literature on milk cessation methods clearly emphasizes the importance of milk yield at dry-off on udder health and cow comfort after dry-off. No published studies. however, have specifically investigated the optimal milk production level at drv-off for best future udder health and welfare of the cow. Based on the existing literature, a target of 15 kg/day of milk or less at dry-off is recommended. A 5- to 7-day intervention of intermittent milking (e.g., 1x milking/day) prior to dry-off, implemented with or without changes in feeding, will adequately lower milk yield to accelerate involution and enhance udder health and cow comfort. Because management conditions and facilities vary among farms, herd managers should develop individualized protocols for drying off in consultation with their veterinarians, always remembering the importance of a clean, dry, comfortable environment for dry cows. Most studies of drying-off practices have been carried out using antibiotic DCT in all cows. Considering concerns about increasing levels of antimicrobial resistance and pressure to reduce antimicrobial use, the effects of different methods to stop milk production on udder health when using selective DCT warrants further research.

### **Conflict of interest statement**

The authors of this paper have no financial or personal relationship with other individuals or organisations that could inappropriately influence or bias the content of this paper.

### References

- Agenäs, S., Dahlborn, K., Holtenius, K., 2003. Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. Livestock Production Science 83, 153–164.
- Bach, A., De-Prado, A., Aris, A., 2015. The effects of cabergoline administration at dry-off of lactating cows on udder engorgement, milk leakages, and lying behavior. Journal of Dairy Science 98, 7097–7101.
- Bachman, K.C., Schairer, M.L., 2003. Invited review: bovine studies on optimal lengths of dry periods. Journal of Dairy Science 86, 3027–3037.
- Barkema, H.W., von Keyserlingk, M.A.G., Kastelic, J.P., Lam, T.J.G.M., Luby, C., Roy, J.P., LeBlanc, S.J., Keefe, G.P., Kelton, D.F., 2015. Invited review: changes in the dairy industry affecting dairy cattle health and welfare. Journal of Dairy Science 98, 7426–7445.
- Bertulat, S., Fischer-Tenhagen, C., Suthar, V., Möstl, E., Isaka, N., Heuwieser, W., 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. Journal of Dairy Science 96, 3774–3787.

- Bertulat, S., Fischer-Tenhagen, C., Heuwieser, W., 2015. A survey of drying-off practices on commercial dairy farms in northern Germany and a comparison to science-based recommendations. Veterinary Record Open 2, e000068.
- Bertulat, S., Isaka, N., de Prado, A., Lopez, A., Hetreau, T., Heuwieser, W., 2017. Effect of a single injection of cabergoline at dry off on udder characteristics in highyielding dairy cows. Journal of Dairy Science 100, 3220–3232.
- Boogaard, B.K., Oosting, S.J., Bock, B.B., 2008. Defining sustainability as a sociocultural concept: citizen panels visiting dairy farms in the Netherlands. Livestock Science 117, 24–33.
- Boutinaud, M., Isaka, N., Lollivier, V., Dessauge, F., Gandemer, E., Lamberton, P., De Prado Taranilla, A.I., Deflandre, A., Sordillo, L.M., 2016. Cabergoline inhibits prolactin secretion and accelerates involution in dairy cows after dry-off. Journal of Dairy Science 99, 5707–5718.
- Bradley, A.J., Green, M.J., 2004. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. Veterinary Clinics of North America Food Animal Practice 20, 547–568.
- Bushe, T., Oliver, S.P., 1987. Natural protective factors in bovine mammary secretions following different methods of milk cessation. Journal of Dairy Science 70, 696– 704.
- Capuco, A.V., Akers, R.M., 1999. Mammary involution in dairy animals. Journal of Mammary Gland Biology and Neoplasia 4, 137–144.
- Chapinal, N., Zobel, G., Painter, K., Leslie, K.E., 2014. Changes in lying behavior after abrupt cessation of milking and regrouping at dry-off in freestall-housed cows: a case study. Journal of Veterinary Behavior 9, 364–369.
- Comalli, M.P., Eberhart, R.J., Griel, L.C., Rothenbacher, H., 1984. Changes in the microscopic anatomy of the bovine teat canal during mammary involution. American Journal of Veterinary Research 45, 2236–2242.
- Cunningham, J.C., Klein, B.G., 2007. Reproduction and lactation, Textbook of Veterinary Physiology. fourth edn. Saunders Elsevier, St. Louis, MO, USA, pp. 501–516.
- Dancy, K.M., Ribeiro, E.S., DeVries, T.J., 2019. Effect of dietary transition at dry off on the behaviour and physiology of dairy cows. Journal of Dairy Science 102, 4387– 4402.
- Dingwell, R.T., Leslie, K.E., Schukken, Y.H., Sargeant, J.M., Timms, L.L., Duffield, T.F., Keefe, G.P., Kelton, D.F., Lissemore, K.D., Conklin, J., 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Preventive Veterinary Medicine 63, 75–89.
- Eberhart, R.J., 1986. Management of dry cows to reduce mastitis. Journal of Dairy Science 69, 1721–1732.
- Fujiwara, M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D., 2018. Survey of dry cow management on UK commercial dairy farms. The Veterinary Record 183, 297.
- Gott, P.N., Rajala-Schultz, P.J., Schuenemann, G.M., Proudfoot, K.L., Hogan, J.S., 2016. Intramammary infections and milk leakage following gradual or abrupt cessation of milking, Journal of Dairy Science 99, 4005–4017.
- Gott, P.N., Rajala-Schultz, P.J., Schuenemann, G.M., Proudfoot, K.L., Hogan, J.S., 2017. Effect of gradual or abrupt cessation of milking at dry off on milk yield and somatic cell score in the subsequent lactation. Journal of Dairy Science 100, 2080–2089.
- Green, M.J., Bradley, A.J., Medley, G.F., Browne, W.J., 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. Journal of Dairy Science 91, 1403–1415.
- Halasa, T., Østerås, O., Hogeveen, H., van Werven, T., Nielen, M., 2009. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. Journal of Dairy Science 92, 3134–3149.
- Hop, G.E., de Prado-Taranilla, A.I., Isaka, N., Ocak, M., Bertet, J., Supre, K., Velthuis, A., Schukken, Y.H., Deflandre, A., 2019. Efficacy of cabergoline in a double-blind randomized clinical trial on milk leakage reduction at drying-off and new intramammary infections across the dry period and postcalving. Journal of Dairy Science 102, 11670–11680.
- Hurley, W.L., 1989. Mammary gland function during involution and the declining phase of lactation. Journal of Dairy Science 72, 1637–1646.
- Kelly, A.L., Reid, S., Joyce, P., Meaney, W.J., Foley, J., 1998. Effect of decreased milking frequency of cows in late lactation on milk somatic cell count, polymorphonuclear leucocyte numbers, composition and proteolytic activity. Journal of Dairy Research 65, 365–373.Lacy-Hulbert, S.J., Woolford, M.W., Nicholas, G.D., Prosser, C.G., Stelwagen, K., 1999.
- Lacy-Hulbert, S.J., Woolford, M.W., Nicholas, G.D., Prosser, C.G., Stelwagen, K., 1999. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. Journal Dairy Science 82, 1232–1239.
- Lanctôt, S., Fustier, P., Taherian, A.R., Bisakowski, B., Zhao, X., Lacasse, P., 2017. Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution. Journal of Dairy Science 100, 2269–2281.
- Leitner, G., Jacoby, S., Maltz, E., Silanikove, N., 2007. Casein hydrolyzate intramammary treatment improves the comfort behavior of cows induced into dry-off. Livestock Science 110, 292–297.
- Madouasse, A., Browne, W.J., Huxley, J.N., Toni, F., Bradley, A.J., Green, M.J., 2012. Risk factors for a high somatic cell count at the first milk recording in a large sample of UK dairy herds. Journal of Dairy Science 95, 1873–1884.
- Maynou, G., Elcoso, G., Bubeck, J., Bach, A., 2018. Effects of oral administration of acidogenic boluses at dry-off on performance and behavior of dairy cattle. Journal of Dairy Science 101, 11342–11353.
- Natzke, R.P., Everett, R.W., Bray, D.R., 1975. Effect of drying off practices on mastitis infection. Journal of Dairy Science 58, 1828–1835.
- Neave, F.K., Dodd, F.H., Henriques, E., 1950. Udder infections in the dry period .1. Journal of Dairy Research 17, 37–49.
- Newman, K.A., Rajala-Schultz, P.J., Lakritz, J., DeGraves, F., 2009. Lactoferrin concentrations in bovine milk prior to dry-off. Journal of Dairy Research 76, 426–432.

- Newman, K.A., Rajala-Schultz, P.J., DeGraves, F.J., Lakritz, J., 2010. Association of milk yield and infection status at dry-off with intramammary infections at subsequent calving. Journal of Dairy Research 77, 99–106.
- Nickerson, S.C., 1989. Immunological aspects of mammary involution. Journal of Dairy Science 72, 1665–1678.
- O'Connor, A.M., Sargeant, J.M., 2015. Research synthesis in veterinary science: narrative reviews, systematic reviews and meta-analysis. The Veterinary Journal 206, 261–267.
- Odensten, M.O., Chilliard, Y., Holtenius, K., 2005. Effects of two different feeding strategies during dry-off on metabolism in high-yielding dairy cows. Journal of Dairy Science 88, 2072–2082.
- Odensten, M.O., Berglund, B., Waller, K.P., Holtenius, K., 2007a. Metabolism and udder health at dry-off in cows of different breeds and production levels. Journal of Dairy Science 90, 1417–1428.
- Odensten, M.O., Holtenius, K., Waller, K.P., 2007b. Effects of two different feeding strategies during dry-off on certain health aspects of dairy cows. Journal of Dairy Science 90, 898–907.
- OIE, 2016. The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. https://www.oie.int/fileadmin/Home/eng/Media\_Center/ dayocs/pdf/PortailAMR/EN\_OIE-AMRstrategy.pdf.
- Oliver, S.P., Mitchell, B.A., 1983. Susceptibility of bovine mammary-gland to
- infections during the dry period. Journal of Dairy Science 66, 1162–1166. Oliver, S.P., Sordillo, L.M., 1989. Approaches to the manipulation of mammary involution. Journal of Dairy Science 72, 1647–1664.
- Oliver, J., Dodd, F.H., Neave, F.K., 1956a. Udder infections in the 'dry period' III. The method of drying-off cows at the end of lactation. Journal of Dairy Research 23, 197–203.
- Oliver, J., Dodd, F.H., Neave, F.K., 1956b. Udder infections in the 'dry period' IV. The relationship between the new infection rate in the early dry period and the daily milk yield at drying-off when lactation was ended by either intermittent or abrupt cessation of milking. Journal of Dairy Research 23, 204–211.
- Oliver, S.P., Shull, E.P., Dowlen, H.H., 1990. Influence of different methods of milk cessation on intramammary infections during the periparturient period. International Symposium on Bovine Mastitis, Indianapolis, IN, USA, pp. 92–97.
- Ollier, S., Zhao, X., Lacasse, P., 2013. Effect of prolactin-release inhibition on milk production and mammary gland involution at drying-off in cows. Journal of Dairy Science 96, 335–343.
- Ollier, S., Zhao, X., Lacasse, P., 2014. Effects of feed restriction and prolactin-release inhibition at drying off on metabolism and mammary gland involution in cows. Journal of Dairy Science 97, 4942–4954.
- Pezeshki, A., Capuco, A.V., De Spiegeleer, B., Peelman, L., Stevens, M., Collier, R.J., Burvenich, C., 2010. An integrated view on how the management of the dry period length of lactating cows could affect mammary biology and defence. Journal of Animal Physiology and Animal Nutrition 94, e7-e30.
- Ponchon, B., Lacasse, P., Silanikove, N., Ollier, S., Zhao, X., 2014. Effects of intramammary infusions of casein hydrolysate, ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N' -tetraacetic acid, and lactose at drying-off on mammary gland involution. Journal of Dairy Science 97, 779–788.
- Rajala-Schultz, P.J., Hogan, J.S., Smith, K.L., 2005. Association between milk yield at dry-off and probability of intramammary infections at calving. Journal of Dairy Science 88, 577–579.
- Rajala-Schultz, P.J., Gott, P.N., Proudfoot, K.L., Schuenemann, G.M., 2018. Effect of milk cessation method at dry-off on behavioral activity of dairy cows. Journal of Dairy Science 101, 3261–3270.
- Robert, A., Seegers, H., Bareille, N., 2006. Incidence of intramammary infections during the dry period without or with antibiotic treatment in dairy cows-a quantitative analysis of published data. Veterinary Research 37, 25–48.
- Rutherford, K.M.D., 2002. Assessing pain in animals. Animal Welfare 11, 31–53.
- Schukken, Y.H., Vanvliet, J., Vandegeer, D., Grommers, F.J., 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. Journal of Dairy Science 76, 2925–2930.
- Senel, S., McClure, S.J., 2004. Potential applications of chitosan in veterinary medicine. Advanced Drug Delivery Reviews 56, 1467–1480.

- Shamay, A., Shapiro, F., Leitner, G., Silanikove, N., 2003. Infusions of casein hydrolyzates into the mammary gland disrupt tight junction integrity and induce involution in cows. Journal of Dairy Science 86, 1250–1258.
- Silanikove, N., 2014. Natural and abrupt involution of the mammary gland affects differently the metabolic and health consequences of weaning. Life Sciences 102, 10–15.
- Silanikove, N., Merin, U., Shapiro, F., Leitner, G., 2013. Early mammary gland metabolic and immune responses during natural-like and forceful drying-off in high-yielding dairy cows. Journal of Dairy Science 96, 400–6411.
  Smith, K.L., Todhunter, D.A., Schoenberger, P.S., 1985. Environmental pathogens and
- intramamary infection during the dry period. Journal of Dairy Science 68, 402–417. Sordillo, L.M., Nickerson, S.C., 1988. Morphologic changes in the bovine mammary gland using involution and lactogenesis. American Journal of Veterinary
- Research 49, 1112–1120. Sordillo, L.M., Streicher, K.L., 2002. Mammary gland immunity and mastitis
- susceptibility. Journal of Mammary Gland Biology and Neoplasia 7, 135–146. Sordillo, L.M., Shafer-Weaver, K., DeRosa, D., 1997. Immunobiology of the mammary
- gland. Journal of Dairy Science 80, 1851–1865. Stefanon, B., Colitti, M., Gabai, G., Knight, C.H., Wilde, C.J., 2002. Mammary apoptosis
- and lactation persistency in dairy animals. Journal of Dairy Research 69, 37–52. Stefanowska, J., Plavsic, M., Ipema, A.H., Hendriks, M.M.W.B., 2000. The effect of
- omitted milking on the behaviour of cows in the context of cluster attachment failure during automatic milking. Applied Animal Behaviour Science 67, 277–291. Stelwagen, K., Davis, S.R., Farr, V.C., Eichler, S.J., Politis, I., 1994. Effect of once daily
- milking and concurrent somatotropin on mammary tight junction permeability and yield of cows. Journal of Dairy Science 77, 2994–3001.
- Summers, E.L., Lacy-Hulbert, S.J., Williamson, J.H., Sugar, B.P., 2004. Influence of feeding level after drying off on incidence of mastitis and keratin plug formation in dairy cows. Proceedings of the New Zealand Society of Animal Production, New Zealand. Pp, pp. 48–52.
- Tucker, C.B., Lacy-Hulbert, S.J., Webster, J.R., 2009. Effect of milking frequency and feeding level before and after dry off on dairy cattle behavior and udder characteristics. Journal of Dairy Science 92, 3194–3203.
- Valizaheh, R., Veira, D.M., von Keyserlingk, M.A.G., 2008. Behavioural responses by dairy cows provided two hays of contrasting quality at dry-off. Applied Animal Behaviour Science 109, 190–200.
- van Knegsel, A.T.M., van der Drift, S.G.A., Čermáková, J., Kemp, B., 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: a systematic review. The Veterinary Journal 198, 707–713.
- Vanhonacker, F., Verbeke, W., Van Poucke, E., Pieniak, Z., Nijs, G., Tuyttens, F., 2012. The concept of farm animal welfare: citizen perceptions and stakeholder opinion in Flanders, Belgium. Journal of Agricultural & Environmental Ethics 25, 79–101.
- Vilar, M.J., Hovinen, M., Simojoki, H., Rajala-Schultz, P.J., 2018. Short communication: drying-off practices and use of dry cow therapy in Finnish dairy herds. Journal of Dairy Science 101, 7487–7493.
- Vitale, A.F., Tenucci, M., Papini, M., Lovari, S., 1986. Social-behavior of the calves of semiwild Maremma cattle, *Bos primigenius taurus*. Applied Animal Behaviour Science 16, 217–231.
- von Keyserlingk, M.A.G., Rushen, J., de Passillé, A.M., Weary, D.M., 2009. Invited review: the welfare of dairy cattle - key concepts and the role of science. Journal of Dairy Science 92, 4101–4111.
- Watts, J.M., Stookey, J.M., 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. Applied Animal Behaviour Science 67, 15–33.
- Wayne, R., Macy, H., 1933. The effect of various methods for drying up cows on the bacterial and cell content of milk. Journal of Dairy Science 16, 79–91.
- Zobel, G., Leslie, K., Weary, D.M., Von Keyserlingk, M.A.G., 2013. Gradual cessation of milking reduces milk leakage and motivation to be milked in dairy cows at dryoff. Journal of Dairy Science 96, 5064–5071.
   Zobel, G., Weary, D.M., Leslie, K.E., Von Keyserlingk, M.A.G., 2015. Invited review:
- Zobel, G., Weary, D.M., Leslie, K.E., Von Keyserlingk, M.A.G., 2015. Invited review: cessation of lactation: effects on animal welfare. Journal of Dairy Science 98, 8263– 8277.