Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/j.1439-0396.2010.00991.x

REVIEW ARTICLE

An integrated view on how the management of the dry period length of lactating cows could affect mammary biology and defence

A. Pezeshki[1], A. V. Capuco[1,2], B. De Spiegeleer[3], L. Peelman[4], M. Stevens[1], R. J. Collier[5] and C. Burvenich[1]

1 Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,
2 Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville, MD, USA,
3 Drug Quality and Registration (DruQuaR) Group, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium,
4 Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, and
5 Department of Animal Sciences, The College of Agriculture and Life Sciences, University of Arizona, Tucson, AZ, USA

Keywords
dry period length, bovine mammary gland involution, bovine mammary defence, immune status, intramammary infection

Correspondence
Christian Burvenich, Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium. Tel: +32 (0) 9 264 73 21; Fax: +32 (0) 9 264 74 99;
E-mail: christian.burvenich@ugent.be

Received: 29 September 2009;
accepted: 19 November 2009

Summary

The dry period is necessary to facilitate cell turnover in the bovine mammary gland and to optimize milk production in the next lactation. An 8-week dry period has long been the golden standard of management for dairy cows. Genetic improvements and new management technologies have led to higher milk production and a need for re-evaluation of the dry period length. Over the last decade, dry period length has been proposed to be shortened or eliminated mainly from an economic point of view. However, the influence of modified dry period length on the immune defence of the bovine mammary gland and the occurrence of new intramammary infections has not yet been appreciated. The objective of this review is to discuss the bovine mammary gland biology, defence and systemic health when the dry period length is modified. Shortening or eliminating the dry period may minimize or remove the impact of milk accumulation at dry off, thereby lessening the immunodeficiency of the dam that is characteristic of this period. Composition of mammary secretions may change and the extent of tissue remodelling may be reduced when the dry period is reduced or eliminated. Additionally, impact of the dry period length on energy and nutritional status, and on hormonal and local regulatory factors, lead us to hypothesize that changing the dry period length might also affect the response to intramammary infection. It is concluded that there is a need to integrate mammary gland biology and defence mechanisms in studies dealing with modified dry period lengths.

Introduction

In dairy cows, the dry period is necessary to replace senescent mammary epithelial cells (MEC) and to maximize milk production in the ensuing lactation (Hurley, 1989; Capuco et al., 1997). An 8-week dry period has been a management constant for most dairy farms since World War II (Bachman and Schairer, 2003). Recently, the length of the dry period was re-evaluated (Grummer and Rastani, 2004). Prominent among the reasons to change dry period length (DPL) is the high milk production of our modern dairy cows compared with the production of cows upon which conclusions regarding DPL were initially based.

Although shortening the dry period has been reported to cause negligible milk production loss (Gulay et al., 2003; Schairer, 2001; Bachman, 2002; Annen et al., 2004a), the majority of recent studies have been consistent with past research and shown that milk yield is reduced in cows with short dry periods (SDP) (Madsen et al., 2004; Gulay et al., 2005; Kuhn et al., 2005, 2007; Rastani et al., 2005; Kuhn et al., 2006; Pezeshki et al., 2007, 2008; Church et al., 2008; Gallo et al., 2008; Watters et al., 2008). Completely eliminating the dry period resulted in reduced milk production in the subsequent lactation (Annen et al., 2004b, 2007; Andersen et al., 2005; Rastani et al., 2005; Fitzgerald et al., 2007). Mammary involution and growth occur during the dry period. These physiological phenomena, together with the animal's energy status, are generally accepted to be important factors for milk production efficiency during subsequent lactation. However, mammary gland involution and growth during dry period in SDP cows is poorly understood. The first half of this review will focus on mammary gland involution and energy balance of modern dairy cows with modified DPL during the dry period.

The dry period is also an important time to control intramammary infections (IMI): (i) at drying off antibiotics are intramammarily infused to protect the gland, (ii) there is the physiological clearing of many bacteria during the mid dry period (after drying off), (iii) for management reasons the dry period is an ideal period to treat IMI, and (iv) it is known that many clinical coliform mastitis cases that occur during early lactation originate from new IMI at the end of the dry period (colostrogenesis) (Smith et al., 1985b). These few examples emphasize the need to consider the impact of DPL, not only on mammogenesis and future milk production, but also on udder health (Annen et al., 2004a). Curiously, these phenomena have been studied by lactation physiologists and mastitis workers, respectively, and have not been linked. For example, studies conducted during the last decade for re-evaluation of DPL did not evaluate the impact of shortened or eliminated dry periods on mammary and systemic health.

However, experimental evidence suggests that mammary defence may be affected by modifying the DPL: (i) Poorer quality of colostrum for calves of continuous milking (CM) cows has been reported in some studies and it indicates that the colostrogenesis period is not sufficient for gamma globulin accumulation in these cows (Remond et al., 1997a). (ii) Bovine mammary glands are markedly protected to IMI during mid dry period when fluid volume is considerably reduced (Neave et al., 1950). On the other hand, the incidence of new IMI is increased when milk accumulates in the glands, i.e. during very early (dry off) and late (colostrogenesis) phases of the dry period. During this period there is a decline in the efficacy of lactoferrin and phagocytic activity is impaired by the accumulation of milk components (Burvenich et al., 2007) and accumulated casein and milk can promote microbial growth. (iii) DPL impacts the amount of milk accumulated in the gland at dry off and this is inversely related to mastitis resistance. The mammary gland reduces its capacity to secrete milk in response to IMI (Harmon, 1994) and we suggest that down-regulation of genes involved in milk secretion is a well-conserved evolutionary autodefence mechanism that enables the lactating animal to combat invading pathogens more efficiently (Rinaldi et al., 2009). (iv) Reversible pharmacological cessation of milk secretion (e.g. by use of colchicine or endotoxin) can improve the cure rate of IMI by hastening the drying off (Patton, 1974; Burvenich and Peeters, 1980; Oliver and Smith, 1982a,b). (v) The increased yield at drying off has been associated with an increased risk of new IMI in the dry period and calving, mainly because of increased risk of leaking milk and intramammary pressure, and level of milk components (Huxley et al., 2002; Bradley and Green, 2004; Rajala-Schultz et al., 2005). The amount of milk accumulation and its resorption are influenced by DPL. The slow transition from drying off to the mid dry period delays the protective characteristics of this period while milk components level remains high interfering with leucocyte functions (Sordillo and Nickerson, 1988). Because DPL influences the kinetics of involution and the volume and composition of mammary secretions, it may significantly impact susceptibility to new IMI during the dry period.

During colostrogenesis, calving and early lactation, dairy cows experience many endocrine, nutritional and metabolic changes (Goff and Horst, 1997; Goff et al., 2002). During this transition period, factors that are known to influence immune function could be affected by DPL (Pezeshki et al., 2007). The purpose of this review is to discuss mammary gland biology and defence when DPL is modified in the light of indirect findings from the previous research.

Impact of the dry period on milk production in dairy cows: hypothesis

Four hypotheses have been proposed to explain the need for a non-lactating period between successive lactations in dairy cows (Swanson, 1965; Smith et al., 1967; Swanson et al., 1967; Capuco et al.,

1997). The first hypothesis is nutritionally based, and suggests that a dry period is required for cows to have sufficient body reserves before calving to support optimal milk production in the subsequent lactation. Determination of DPL based on productivity and body condition first was proposed by Woodward and Dawson in 1926 [cited by Arnold and Becker (1936)]. Afterwards, Dickerson and Chapman (1939) reported that a pronounced reduction in milk yield was observed in undernourished cows with SDP. This hypothesis was subsequently disproven based upon results of between cow and within cow (within udder) studies. Even if cows exhibited improved body weight, lower milk production was observed with zero or 30-day DPL compared with 60-day DPL (Swanson, 1965; Lotan and Alder, 1976). Furthermore, in a half-udder study, reduced milk yield for CM quarters was observed despite equal nutrient availability to all quarters (Smith et al., 1967). The second hypothesis is hormonally based, and proposes that reduced milk production in cows with short or no dry periods is resulting from continuous influence of galactopoietic hormones. This hypothesis was disproved by Smith et al. (1967) who, utilizing a within-udder design, demonstrated reduced milk yield in CM quarters compared with control quarters, despite exposure of all quarters to the same endocrine milieu. The third hypothesis was based on cell number, suggesting reduced MEC number as a cause for depressed milk yield in cows with modified DPL. This was invalidated (Swanson et al., 1967; Capuco et al., 1997), as no differences in dry fat-free tissue weight, DNA concentration, total DNA content, or the number of alveoli per tissue section was observed in quarters with 6-week differences in DPL. These authors, therefore, suggested that reduced milk in CM quarters can be attributed to decreased secretory activity per unit of mammary secretory tissue and physiological factors affecting the cells during lactogenesis, rather than systemic hormonal regulation or MEC numbers. Using [^{3}H]-thymidine incorporation to evaluate mammary cell proliferation, Capuco et al. (1997) demonstrated 80% greater incorporation in mammary tissue from control (60-day dry) cows compared with CM cows. They also reported that total mammary DNA content increased twofold from 53 to 7 days prepartum but was not affected by lactation status. Therefore, a fourth hypothesis was proposed, suggesting that a dry period of appropriate length was necessary for promoting cell turnover and replacement of senescent MEC during late gestation (Capuco et al., 1997).

Historical studies and current re-evaluations of dry period length

A dry period has been used on dairy farms as a management practice since the 1800 (Arnold and Becker, 1936). At that time, the optimum length of the dry period was a controversial subject among farmers (Annen and Collier, 2005). However, in the early 1900s the complete cessation of milking in the seventh month of pregnancy was adapted as a common practice for maximizing milk production in the ensuing lactation (Arnold and Becker, 1936). The DPL ranging from 4 to 10 weeks was recommended by many widely used textbooks in dairy husbandry between 1911 and 1930 (Arnold and Becker, 1936). Depending on the physical condition of the cow, a 6- to 8-week DPL favoured in the majority of the texts. The standard practice of drying off the cows, 60 days before parturition, was developed from dairymen experience. Spurred by the shortage of food during World War II, a 305-day lactation and 60-day dry period first was adopted in the United Kingdom (Knight, 1998) and then in the Unites States (Annen et al., 2004b). Bachman and Schairer (2003) summarized the results of studies on DPL between 1936 and 1996. They reported that best milk yield in the next lactation was obtained when DPL was 40–60 days (e.g. Coppock et al., 1974; Dias and Allaire, 1982; Funk et al., 1987; Rémond et al., 1992; Makuza and McDaniel, 1996).

In recent years, it has been considered important to re-evaluate DPL primarily for the following reasons: (i) most historic studies involved retrospective analysis of farm data (e.g. DHI) in which milk yield was regressed against days dry, rather than utilizing designed animal experiments (Bachman and Schairer, 2003; Grummer and Rastani, 2004, 2005; Kuhn and Hutchison, 2005), (ii) DPL in older studies was confounded by reproduction and management issues. Cows that had been exposed to SDP were frequently in that category because of early calving for a variety of reasons (e.g. carrying twins, spontaneous abortions, missed breeding or dry off dates), none of which has a positive impact on milk production in the next lactation (Bachman and Schairer, 2003), (iii) cow numbers used in older designed studies were frequently insufficient to detect small changes in milk yield, (iv) there was limited information regarding the impact of DPL for today's high-producing dairy cows. Modern dairy cows produce much more milk throughout lactation and at dry-off than cows from 30 years ago, (v) Widespread use of new management technologies

and practices, such as bovine somatotropin (ST), increased milking frequency, total mixed ration, and photoperiod management may impact the efficacy of shortened or omitted dry periods. In contrast to rat and mouse, which initiate significant mammary involution within 24 h of forced weaning, bovine mammary involution is slower and partially reversible after 11 days of milk stasis (Noble and Hurley, 1999). Because mammary involution in dairy cows is complete by Day 25 of the dry period and significant proliferation occurs by this time (Capuco et al., 1997), a 30-day dry period may be adequate time for involution and tissue regeneration under appropriate circumstances. Therefore, length of the dry period has become an active area of research and shortening the dry period to <60 days or eliminating the dry period have been promoted (e.g. Remond et al., 1997b; Bachman, 2002; Annen et al., 2004a, 2007; Andersen et al., 2005; Rastani et al., 2005; Fitzgerald et al., 2007; Pezeshki et al., 2007, 2008).

Bovine mammary gland involution and dry period length

Mammary gland involution during the dry period

Mammary involution can be induced at any stage of lactation by terminating milk removal. It appears that the response to induced involution in ruminant mammary tissue is slower than that in rodents. For example, within 24 h of litter removal, mRNA abundance of α-, β- and γ-casein was observed to fall by up to 95% in rat mammary tissue (Travers et al., 1996). Abundance of casein and α-lactalbumin mRNA was also reduced in bovine mammary tissue 3 days after cessation of milk removal, but to a lesser extent than in rodents (Goodman and Schanbacher, 1991). However, after 7 days, α_{S1}-casein and α-lactalbumin mRNA was reduced by 85% and 99% respectively (Wilde et al., 1997). The morphological changes in mammary tissue during the dry period in dairy animals are less pronounced and distinctly different compared with those seen during mammary involution in non-pregnant rodents. Morphological changes during the dry period in dairy cows more strictly reflect a change in the secretory state of the mammary gland rather than characteristics of cell loss and tissue regression. Alveolar structure of bovine mammary tissue generally remains intact throughout a typical dry period (Holst et al., 1987; Wilde et al., 1997). Luminal area in mammary tissue reaches a minimum on Day 25 of dry period, whereas stromal area is maximum at the same time in dairy cows (Capuco et al., 1997). It has been shown that MEC in ruminants do not regress to the same extent as occurs in rodents mammary gland and apparently some synthetic and secretory activity of these cells are maintained throughout the dry period (Holst et al., 1987; Sordillo, 1987; Sordillo and Nickerson, 1988). Sloughing of apoptotic epithelial cells into the alveolar lumen and detachment from the basement membrane observed during first 2 weeks of involution in rat and mice is not detectable during dry period in dairy cows (Sordillo and Nickerson, 1988; Hurley, 1989; Capuco et al., 1997). It has been reported that indices of both proliferation and apoptosis are increased within the first 10 days of the dry period (Capuco et al., 2006). Apoptosis occurs in the bovine mammary gland after milk cessation (Quarrie et al., 1996; Wilde et al., 1997). In dairy cows, apoptosis increases to a peak during the first 72 h after dry-off (Annen and Collier, 2005) and continues throughout late gestation and into early lactation, reaching a second peak during the first week after parturition (Annen et al., 2007). Mammary epithelial cell proliferation increases at or shortly after the initial increase in apoptosis (Annen and Collier, 2005). Similarly, proliferation of MEC increased in non-suckled glands 5–7 days after cessation of suckling in beef cows (Capuco and Akers, 1990). Although mammary cell loss does not occur extensively during a standard dry period, tissue remodelling including changes in cell populations, alveolar structure and syntheses of extracellular matrix occur extensively (Holst et al., 1987; Hurley, 1989; Capuco et al., 1997). As a conclusion, extensive cell turnover occurs during the dry period and the number of epithelial cells rapidly increases during the last 2 months of gestation (Capuco et al., 1997).

With normal management of dairy cows there is an overlap of lactation and pregnancy. These animals are also pregnant at cessation of milk removal. Most likely, increased mammary cell turnover during dry period is a consequence of concomitant pregnancy and milk removal (Capuco et al., 2006). Mammogenic and lactogenic stimulation of pregnancy tends to counterbalance the apoptotic effects of accumulated milk at dry off by enhancing cell proliferation and inhibiting apoptosis (Capuco et al., 2006). Consequently, concomitant pregnancy opposes stimuli for mammary involution during dry period. Similarly, simultaneous pregnancy retards mammary gland involution after forced weaning in mice (Capuco et al., 2002). Conversely, the mammary gland of non-pregnant dairy cows may undergo extensive destruction of the lobular–alveolar

structure after dry-off, as expected in stage II of involution (Leitner et al., 2007). Forced weaning of sheep at 5 days of lactation induced involution which was complete after 30 days (Tatarczuch et al., 1977). In species that are not pregnant at cessation of milk removal, extensive and rapid cell loss occurs in the mammary gland, followed by gland remodelling to structural similarity to a virgin gland (Capuco and Akers, 1999; Capuco et al., 2002). Consequently, the process of cell renewal and tissue remodelling that occurs following dry-off with concomitant pregnancy, has been descriptively termed 'regenerative involution' (Capuco et al., 2003).

Mammary epithelial cell proliferation and apoptosis are controlled by systemic and local factors after cessation of milk removal. Yet there is a general lack of research on specific factors involved in local regulation of bovine mammary gland apoptosis and involution. Based on data obtained in rodents, the major systemic effects are exerted by the galactopoietic and lactogenic hormones, prolactin, growth hormone, insulin-like growth factors (Wilde et al., 1999) and by systemic glucocorticoid and progesterone, which can inhibit stage II involution (Ongsakul et al., 1985; Feng et al., 1995). The level of systemic lactogenic hormones drops immediately after milking cessation *in vivo* (Lamote et al., 2004b). Importance of the absence of prolactin and ST for mammary gland involution has been shown in rodents (Marti et al., 1999). This has also been demonstrated for cows *in vitro* (Accorsi et al., 2002). It is known that in ruminants prolactin only plays a role in the induction of lactation, while the galactopoiesis in this species is controlled by ST [119]. However, in other species like rodents and humans the dominant role for galactopoiesis and lactogenesis is played by prolactin rather than ST (Lamote et al., 2004b). It has been demonstrated that reduction in circulating concentration of prolactin accelerates the mammary involution (Wilde et al., 1999). The expression of Insulin-like growth-factor binding protein 5 (IGFBP-5), which antagonizes the survival effects of IGF-I, is repressed in the presence of prolactin, growth hormone and IGF-I (Wilde et al., 1999). IGFBP-5, which impacts the availability of IGF-I to target tissues, is secreted locally by MEC. Prolactin reduction caused by cessation of milk removal reduces its inhibition of IGFBP-5 expression by epithelial cells and promotes apoptosis by decreasing the survival activity of IGF-I (Wilde et al., 1997). Additionally, IGF-I stimulates MEC proliferation *in vitro* (McGrath et al., 1991) and *in vivo* (Collier et al., 1993). The role of ST is thought to be managed through elevation of IGF-I (Annen et al., 2004b). It should be noted that this information regarding systemic control of mammary gland involution and apoptosis is based on findings in rodents. Apoptosis can be induced by milk stasis in rodent mammary tissue (Quarrie et al., 1996). Local control of the MEC apoptosis occurs following accumulation of the local factors in stored milk and/or MEC and because of physical distension on the mammary epithelium after milk stasis (Wilde et al., 1999). IGFBP-5 and transforming growth factor-β1 (TGF-β1) are among the known local factors which are elevated in stored milk after milk cessation (Wilde et al., 1999). The role of TGF-β1 is not very well understood, but it is upregulated when apoptosis occurs both *in vitro* and *in vivo* (Wilde et al., 1999). It was beyond the scope of this review to discuss the mechanisms of survival and apoptosis in the mammary gland by local growth factors [reviewed by Lamote et al. (2004b)]. Apoptosis may be stimulated by physical distension through disrupting cell shape. It appears that perturbation of mammary cell shape in culture changes intramammary composition, intracellular free-calcium content and expression of genes with a role in cell fate and morphological development in some embryonic and adult tissue (Huguet et al., 1995; Wilde et al., 1999). Local control of apoptosis can overcome the anti-apoptotic effects of galactopoietic hormones. In addition to interruption of systemic lactogenic hormones, immediately after cessation of milking there is also a decrease in galactopoietic hormone levels (Vangroenweghe et al., 2005). It is suggested that this interruption in hormonal secretions can lead to a rapid decline in milk secretion or down-regulation of differentiated gene expression in rodents (Vangroenweghe et al., 2005). Thus, systemic hormones, local factors, pregnancy and stage of lactation are factors that can affect the rate of mammary gland involution (Capuco et al., 2002).

Mammary gland involution in cows with modified dry period lengths

Although some recent studies have demonstrated small (3.6%) or no decrease in milk production following SDP with or without bovine ST supplementation in dairy cow (Schairer, 2001; Bachman, 2002; Gulay et al., 2003, 2004), most recent studies, support previous demonstration of milk yield depression in cows with SDP (Madsen et al., 2004; Gulay et al., 2005; Kuhn et al., 2005, 2007; Rastani et al., 2005; Pezeshki et al., 2007, 2008; Church et al., 2008; Gallo et al., 2008; Watters et al., 2008).

Discrepancies between the studies reporting an effect of SDP on subsequent milk production may arise from a multitude of factors such as, differences in management schemes, genetic potential of milk production, breed, experimental models (between-cow, within-cow or half-udder model) and the number of cows used in a specific study (statistical power). Mammary involution and growth occur during the dry period and the rate of this process is an important factor for milk production efficiency in the next lactation. The previous literature, either between or within cow studies, has only compared mammary gland involution of cows provided a normal dry period or no dry period. To the best of our knowledge, there are no data concerning MEC turnover in cows given SDP. Observed substantial milk production loss during early lactation rather than other stages of lactation (Lotan and Alder, 1976; Gulay et al., 2005; Pezeshki et al., 2008) and reduced half udder size (Gulay et al., 2005) for cows given SDP compared with control animals can provide evidence for less developed mammary glands in these animals. Early events of involution may be inhibited in cows with SDP (Fig. 1). Lower milk production at dry-off of cows with SDP can influence early involution through reduced milk accumulation and induction of local factors (Fig. 1). As previously described, the role of local effects in mammary gland involution can be caused by accumulation of pro-apoptotic factors in the stored milk and physical distention of the mammary epithelium during milk stasis (Wilde et al., 1999) (Fig. 1). Moreover, factors influencing apoptosis and proliferation may be influenced by increasing effects of pregnancy as calving approaches (Fig. 1). Drying off close to parturition to accommodate SDP may result in a greater impact of lactogenic and mammogenic hormones of pregnancy and thus alter the involution and proliferation profile within the mammary gland relative to cows with standard dry periods (Fig. 1).

Although omitting the dry period completly did not affect the milk production of the next lactation either without using bovine ST in dairy cows (Remond and Bonnefoy, 1997) or using bovine ST in multiparous cow (Annen et al., 2004a), in most experiments it has resulted in production losses of 10–40% in the subsequent lactation (Sanders, 1928; Swanson, 1965; Smith et al., 1967; Rémond et al., 1992; Remond et al., 1997a; Annen et al., 2004a, 2007; Andersen et al., 2005; Fitzgerald et al., 2007). We have hypothesized that CM does not allow replacement of senescent and progenitor cells, which could be an explanation for reduced milk yield for these animals in the ensuing lactation (Capuco et al., 1997, 2006). Progenitor cells are responsible for expanding and maintaining the number of mammary secretory cells (Capuco and Akers, 1999). Mammary epithelial cell proliferation is higher in glands that are permitted to have typical dry period than in those continuously milked prepartum (Capuco et al., 1997; Annen et al., 2007). Mammary epithelial cell proliferation is reduced in CM cows throughout the last 35 days of gestation; however, net mammary growth in these animals was not impaired (Capuco et al., 1997). The temporal pattern of apoptosis is different between CM and control glands during the dry period and the apoptotic index is greater in control glands than CM glands during

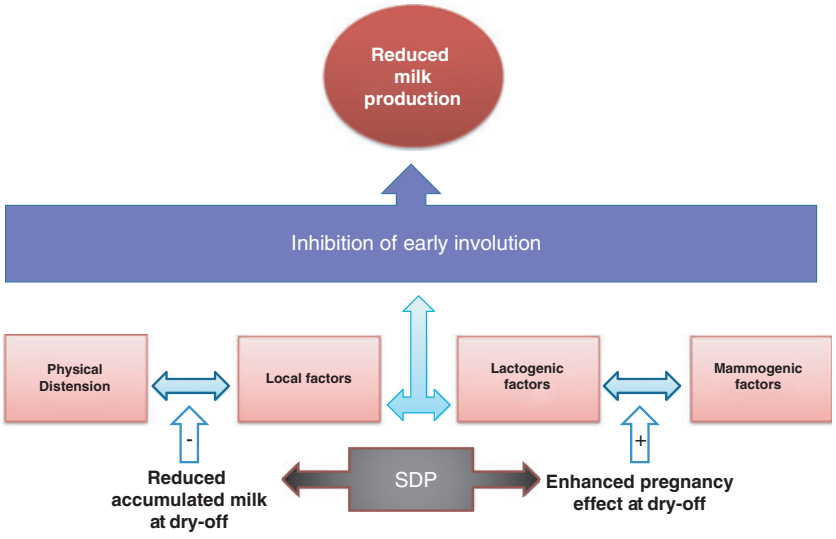

Fig. 1 It is hypothesized that shortening the length of dry period in dairy cows may inhibit early events of involution after milk removal cessation, causing to milk production loss in the subsequent lactation. Early involution may be inhibited through reduced milk accumulation and enhanced pregnancy effect at dry-off in cows subjected to short dry periods (SDP). Pregnancy may inhibit apoptosis and promote proliferation by enhancing the mammogenic and lactogenic stimulations on mammary gland at dry-off. Apoptosis may be inhibited as a result of reduced milk accumulation and stored proapoptotic factors, as well as decreased physical distension of the mammary epithelium at dry off in cows with SDP.

early lactation (Annen et al., 2007). Increased apoptosis during early lactation may provide a mechanism for removal of senescent cells and may reflect the number of new MEC generated during late gestation (Capuco et al., 2006). Reduced apoptosis in CM cows or quarters is most likely attributable to a lack of milk accumulation that provides a local pro-apoptotic stimulus. A reduction in proliferation accompanied by a reduction in apoptosis reduces cell turnover in CM glands and increases carry-over of senescent mammary cells into the ensuing lactation (Capuco et al., 1997). Senescent MEC in the following lactation would have reduced functionality, i.e. less secretory ability and less proliferative capability, and cause reduced milk yield in CM cows or quarters (Capuco and Akers, 1999; Annen et al., 2004b, 2007). Reduced secretory activity per unit of mammary tissue in CM cows was also reported in an early study (Swanson et al., 1967).

Energy status of cows with modified dry period lengths

Changes in diet and grouping of cows during the dry period may lead to increased stress, reduced feed intake and increased metabolic complications postpartum (Grummer and Rastani, 2005). Typical feeding management of cows with a traditional dry period (~60 days) involves two diet changes: far-off and close up diets (Grummer and Rastani, 2005) (Fig. 2). The far-off diet with low-energy density is designed to maintain body condition of the cow and is delivered during first 5 weeks of the dry period (Goff and Horst, 1997) (Fig. 2). Subsequently, the close up diet with moderate-energy density is delivered during the final 3 weeks of the dry period (Grummer and Rastani, 2005) (Fig. 2). It is designed for adaptation of cow and rumen micro-organisms to the high-energy lactation diet provided immediately after parturition. Feeding a single diet throughout the dry period may help reduce the likelihood of stress and its impact on key physiological parameters during the dry period and subsequent lactation. Cows may be over-conditioned and run into increased incidence of metabolic disorders if fed a moderate-energy diet for an 8-week dry period (Rukkwamsuk et al., 1999). Although feeding a low-energy diet during the 8 weeks can be successful, questions persist about the impact of a sudden change from low-energy diet to high-energy diet on fresh cows and the adaptation of rumen microorganism (Grummer and Rastani, 2005). Feeding high-fibre diets increases the population of cellulytic and methane-producing bacteria, which decreases the efficiency of utilizing dietary energy (Johnson and Johnson, 1995). Additionally, length of papillae and the absorptive capacity of the rumen mucosa are reduced on low-energy diets, with as much as 50% of the absorptive area lost during the first 7 weeks of the dry period (Dirksen et al., 1985; Goff and Horst, 1997). Shortening or eliminating the dry period can permit a single, relatively high-energy diet to be fed during late gestation (Grummer and Rastani, 2005) (Fig. 2). Improved dry matter intake, metabolic profiles, body condition score, body weight and mean negative energy balance has been suggested in cows with short (Gulay et al., 2003, Lotan and Alder, 1976; Gulay et al., 2005; Pezeshki et al., 2007, 2008; Watters et al., 2008) or omitted dry periods (Rémond et al., 1992; Andersen et al., 2005; Rastani et al., 2005). Therefore, shortening or eliminating the dry period improves the energy

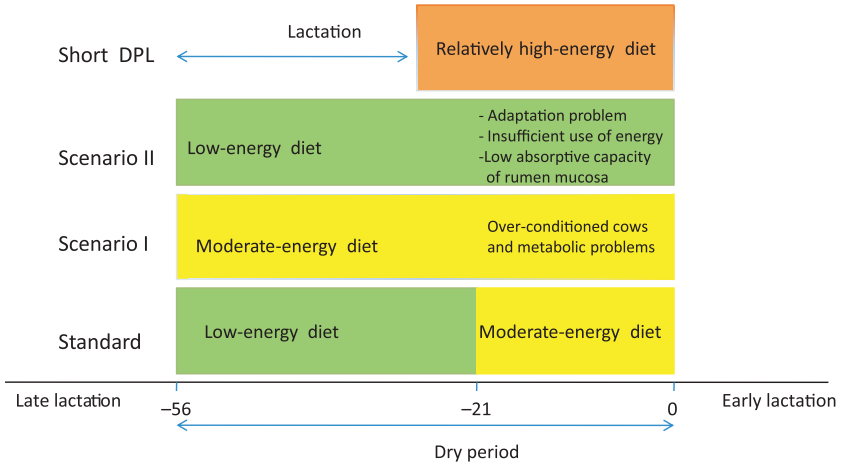

Fig. 2 The schematic illustrates two possible feeding scenarios for minimizing the stress caused by dietary changes during dry period. Feeding the animals with moderate-energy (scenario I) or low-energy diets (scenario II) throughout the dry period may cause to milk production loss in the subsequent lactation. Shortening the length of dry period can be an appropriate management strategy for decreasing the negative energy balance at peripartum minimizing the frequency of dietary changes and feeding relatively high-energy diets; DPL, dry period length.

balance during the final week of gestation and early lactation, removing the nutritional stress of diet change, easing the transition to lactation, and reducing risk of metabolic disease (Pezeshki et al., 2007, 2008) (Fig. 2). However, as mentioned above, most recent literature supports the results of historic studies suggesting that milk production is maximal for cows with 56-day DPL. This further suggests that milk loss in cows with SDP is most likely related to incomplete involution rather than metabolic factors.

Does dry period length affect mammary defence?

Dry period length, composition of secretion and new infections

The importance of the dry period in the dynamics of IMI in dairy cattle has been studied over many years. The dry period is an important focus for mastitis control strategies in dairy herds (Neave et al., 1950; Smith et al., 1985a; Oliver and Sordillo, 1988; Burvenich et al., 2003), because many IMI that occur during the dry period carry into the next lactation and cause clinical mastitis (Hogan and Smith, 2003; Sordillo, 2005). We have recently started developing methods to investigate new aspects of bovine mammary gland defence with analytical techniques during the dry period (De Spiegeleer et al., 2008; Pezeshki et al., 2009). The rate of IMI is not constant across the dry period (Smith et al., 1985a; Bradley and Green, 2004). Clinical and experimental data support the concept that bovine mammary glands are more susceptible to new IMI during the early (drying off) and late (colostrogenesis) dry period than during the remainder of the dry period or lactation (Kehrli and Shuster, 1994; Burvenich et al., 2000, 2007; Sordillo and Streicher, 2002). Increased rates of IMI during transition periods (drying off and near calving) may be attributable to changes in natural protective factors and antibacterial factors, the anatomy and physiology of the teat end, and the extent of exposure to mastitis pathogens (Comalli et al., 1984; Oliver and Sordillo, 1989). To our knowledge, there are no data about composition of mammary secretions during the dry period for cows with SDP, evidence is indirect. Along with changes in mammary histology during the dry period, dramatic changes occur in the composition of mammary secretions (Breau and Oliver, 1985; Bushe and Oliver, 1987; Sordillo and Nickerson, 1988; Athie et al., 1996). Significant changes in cellular and chemical composition of mammary secretions occur during early, mid and late dry period and we hypothesize that modifications of DPL may influence the occurrence of new IMI by altering the composition of mammary secretions during late gestation (Fig. 3).

The secretion of components of both the innate and acquired immune system is elicited immediately after milk removal cessation (Clarkson et al., 2004; Stein et al., 2004). Cell concentrations in milk secretion rise for the first 2 weeks after cessation of milk removal, and then decrease to become stable during much of the period (Jensen and Eberhart, 1981; McDonald and Anderson, 1981a; Miller et al., 1990). In general, numbers of polymorphonuclear (PMN) leucocyte (although there is a difference between

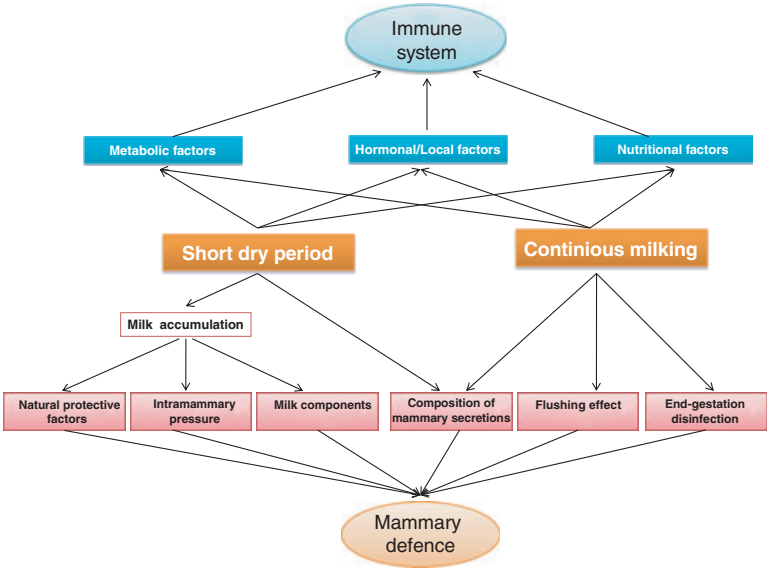

Fig. 3 The schematic summarizes the hypothesis that prepartum cellular immune function and mammary defence may be influenced in cows with short dry period and in continuously milked cows.

PMN and neutrophils, in this review the abbreviation of PMN is used as equivalent for neutrophils), macrophages and lymphocytes, and concentrations of immunoglobulins in mammary secretions remain low during this period (Craven and Williams, 1985; Breau and Oliver, 1986; Hurley and Rejman, 1986). There are some contradictory reports regarding the major cell types in mammary secretions during the early dry period. In one report, macrophages were the major cell type during the early dry period, with PMN being the predominant cell type 2 days after termination of milking, followed by macrophages (McDonald and Anderson, 1981a). In another study, equal frequency of macrophages, PMN and lymphocytes at dry-off were reported, and PMN became the primary cell type 9 days after dry off (Hirsch, 1987). In yet another study, macrophages were the predominant immune cells in secretions from uninfected quarters during first 4 weeks of the dry period, whereas PMN were the major cell type in infected quarters (Jensen and Eberhart, 1981). Concentrations of fat, casein, lactose, citrate, α-lactalbumin and the citrate to lactoferrin molar ratio decrease during the first week of dry period (Akers et al., 1986; Hurley, 1987, 1989; Wilde et al., 1997). Abundance of β-lactoglobulin mRNA in bovine mammary tissue remains unchanged 3 days after cessation of milking (Goodman and Schanbacher, 1991). The concentration of lactose and citrate as the major regulators of osmolarity in mammary secretions undergo major reduction during early dry period in comparison with other components (Hurley and Rejman, 1986; Hurley, 1987). By contrast, there are small and inconsistent changes in concentrations of fat, casein, β-lactoglobulin and α-lactalbumin in mammary gland secretions. The concentrations of proteins in mammary secretions, e.g. serum albumin, and immunoglobulins that are derived from blood, generally increase while there is a reduction in mammary fluid volume during the dry period (Hurley and Rejman, 1986; Hurley, 1987). This is because of relatively increased permeability of MEC tight junctions in this period, permitting passive diffusion of serum proteins into the alveolar lumen. Immunoglobulins in mammary secretions originate locally from cells of the lymphocyte plasma cell series or they are of humoral origin (Lavau et al., 1978). The concentration of all immunoglobulin classes, i.e. IgG_1, IgG_2, IgA and IgM, increase markedly by the first week of the dry period (Watson et al., 1972).

The composition of mammary secretions and morphology of bovine mammary tissue remains relatively constant during the mid dry period (Oliver and Bushe, 1987; Sordillo, 1987). In general, considerable changes in mammary gland structure and function do not occur until parturition (Sordillo, 1987; Sordillo and Nickerson, 1988). Because of markedly reduced fluid volume and accompanying changes in mammary secretion (the major constituents of milk are minimal), the mammary gland is highly resistant to new IMI during the mid dry period (Burvenich et al., 2007), especially to Gram-negative bacteria (Oliver and Mitchell, 1983; Breau and Oliver, 1986). Lactoferrin, the major protein found in mammary secretions during this period, is an important inhibitory component against coliforms (Oliver and Bushe, 1987; Rejman et al., 1989; Goff and Horst, 1997; Burvenich et al., 2007). Additionally, reduced citrate and increased bicarbonate concentrations support the iron-chelating properties of lactoferrin (Rainard and Riollet, 2006). Bicarbonate is required for the binding of iron by lactoferrin and can overcome the deleterious effects of citrate on IMI (discussed later). Citrate is absorbed from the udder to blood, while bicarbonate diffuses from blood to the udder (Reiter et al., 1975; Griffith and Humphrey, 2004). Similarly, immunoglobulin concentrations are elevated during this period, but more moderately than during the early dry period. Lymphocytes appear to be the predominant cell type in uninfected quarters of an involuted udder; however, large quantities of macrophages are also present. The number of PMN generally remains lower than lymphocytes and macrophages during mid dry period.

Selective transport and accumulation of immunoglobulins and the onset of copious secretion of proteins, fat, and carbohydrates, and accumulation of colostrum are main characteristics of lactogenesis–colostrogenesis (Breau and Oliver, 1986; Sordillo and Nickerson, 1988). Because of colostrum accumulation, the intramammary pressure increases. This period most likely begins 15–20 days before parturition (Sordillo and Nickerson, 1988). Colostrogenesis occurs in two stages that can be characterized by both morphological and biochemical changes (Fleet et al., 1975; Oliver and Sordillo, 1988). In the first stage, the volume of precolostral fluid in the mammary gland cistern increases slowly over the last 2 weeks of the dry period. In the second stage which begins immediately prior to parturition, copious colostral secretion occurs (Fleet et al., 1975). The permeability of tight junctions is decreased and synthetic ability of secretory epithelia is increased during the last 2 weeks of gestation (Oliver and Sordillo, 1988). The lower permeability of tight

junctions at calving results in inhibition of paracellular transfer of serum proteins and ions into milk (Nguyen and Neville, 1998). Instead, most transport is via the trans-cellular pathway (Kishimoto et al., 1989; Nguyen and Neville, 1998). Low concentrations of antibacterial components (phagocytes and lactoferrin), somatic cells and serum albumin but high concentrations of casein, lactose, and citrate are characteristic of mammary secretions at this stage (Nonnecke and Smith, 1984; Breau and Oliver, 1986; Oliver and Sordillo, 1988). Immunoglobulin concentration reaches its maximum level in mammary secretions during colostrogenesis, at 5–10 days before calving (Sordillo and Streicher, 2002). Most of the IgG in mammary secretions is of humoral origin, whereas IgA and IgM are produced locally (Lascelles, 1979). Approximately, 90% of all colostral immunoglobulins that are transferred to calves is IgG_1 and IgG_2. Although the concentration of IgG_1 and IgG_2 in the bovine blood is roughly identical (924.3 and 1330.4 mg/dl respectively; Burton et al., 1991a), IgG1 appears in colostrum in much higher concentration than IgG_2 (50–90 and 1.5–2 g/l respectively; Elfstrand et al., 2002) and the high concentration of IgG_1 is a unique property of colostrum. The IgG class, and IgG1 in particular, is selectively transported from the maternal plasma across the blood–milk barrier into the colostrum as parturition approaches, and this process is controlled locally by MEC (Brandon and Lascelles, 1975; Guy et al., 1994). Most likely, the neonatal Fc receptor on the acinar epithelial cell plays an important role in the IgG transport during colostrogenesis in ruminants (Mayer et al., 2005). An important role of IgG_2 for opsonophagocytosis of bacteria by PMN and antibody-dependent PMN cytotoxicity has been suggested (Butler, 1983; Detilleux et al., 1994). An early production of IgM plays a crucial role in resistance to bacterial and protozoal parasites, in complement binding activity and efficient bacterial agglutination reactions (Butler, 1983; Detilleux et al., 1994). The concentration of immunoglobulins falls with the onset of copious secretion and accumulation of secretions in the gland. The lower opsonic activity (which is associated with IgG_2, IgM and complement components in the presence of complement) of mammary secretions during the last week of gestation can be a possible reason for reduced phagocytic capacity of macrophages and PMN and decreased effectiveness of mammary secretions as inhibitors of IMI (Craven and Williams, 1985; Smith et al., 1985a). Moreover, as a result of indiscriminate ingestion of fat and casein, phagocytosis and intracellular bacteriolysis by PMN are likely to be inhibited during the period of colostrogenesis (Russell and Reiter, 1975; Russell et al., 1976; Paape and Guidry, 1977). The molar ratio of citrate:Lf increases by approximately 100-fold over the last few days prepartum. Dramatic increase in concentration of citrate is highly correlated with rapid increase in fluid volume and onset of copious secretion (Peaker and Linzell, 1975). The number of cells in mammary secretions of uninfected quarters gradually decreases during the last 2 weeks of gestation. In contrast to the mid dry period when lymphocytes are the predominant cells (Miller et al., 1990; Rainard and Riollet, 2006), lymphocyte number declines during the periparturient period and macrophages appear to be the most prevalent cell type during the late dry period (Jensen and Eberhart, 1981; McDonald and Anderson, 1981b).

Shortening the DPL can result in reduced milk production at drying off. Therefore, concentrations of fat, casein, lactose, and citrate which are high at dry off and interfere with natural defence (Craven and Williams, 1985; Breau and Oliver, 1986; Hurley and Rejman, 1986), may be reduced during this period for SDP cows (Fig. 3). Phagocytes start ingesting milk fat, casein, and cell debris after diapedesis, which decreases their phagocytic function and induces apoptosis (Burvenich et al., 2007). Degraded epithelial cells and accumulated fat and casein are thought to be heterophagocytosed by macrophages, which enter the mammary tissue and secretion in large numbers during early involution. It appears that there are other factors than fat and casein present in the secretion of the involuting gland that may reduce the phagocytic capabilities of the macrophage and PMN. These factors may also reduce the responsiveness of lymphocytes to antigen stimulation. There is an effective competition between citrate and lactoferrin for iron binding and the resulting iron-citrate complex can be utilized by bacteria (Schanbacher et al., 1993). Additionally, excessive milk volume in the udder at dry-off provides an excellent medium for bacterial growth, which can utilize casein and lactose (Smith et al., 1985c; Breau and Oliver, 1986). There also appear to be secretion components that are either increased in concentration or are produced during the process of involution, which can act as growth stimulants for certain bacteria. Because lower concentrations of natural protective factors are present in mammary secretions from glands producing large quantities of milk during late lactation (Smith et al., 1985c; Breau and Oliver, 1986), shortening the dry period may

increase the concentration of these naturally protective factors via reduced milk production when cows are dried off (Fig. 3). Moreover, increased intramammary pressure caused by accumulation of a large volume of milk, which may cause leakage of milk from the teats and facilitate bacterial penetration of the streak canal, can be minimized by shortening DPL (Cousins et al., 1980; Burvenich et al., 2007) (Fig. 3). A positive correlation between the quantity of milk produced during late lactation and susceptibility to new IMI has been reported by others (Oliver et al., 1956).

We hypothesize that shortening or eliminating the dry period will lessen the occurrence of new IMI. Milking cows until parturition removes the immunodeficiency brought about by milk accumulation when drying off to initiate a dry period (Fig. 3). The literature dealing with the effect of CM on IgG and protein content of colostrum are controversial. A few authors claim that CM negatively impacts IgG and protein content of colostrum (Brandon and Lascelles, 1975; Remond et al., 1997b; Rastani et al., 2005). However, Annen et al. (2004a) did not see any effect of CM on IgG content. Colostrogenesis, in terms of changes in cellular and fluid composition, is not affected by shortening the dry period (Remond et al., 1997b; Annen et al., 2004a; Gulay et al., 2005; Rastani et al., 2005; Watters et al., 2008). The bacterial flushing effect of milking continues uninterrupted when cows are given no dry period (Fig. 3). This is important for removing bacterial invaders colonizing the streak canal, teat cistern, or gland cistern and/or compromised PMN (Paape et al., 1995). Continuous milking may improve the efficiency of the immune system and improve teat end disinfection during late gestation (Fig. 3). The change in number and activity of macrophages, lymphocytes and PMN, the molar ratio of citrate : lactoferrin, the concentration of soluble components such as α-lactalbumn, β-lactoglobulin, albumin, immunoglobulins, proteins, peptides, fat, lactose and caseins during the prepartum period requires additional study in cows with short or omitted dry periods because of the potential impact on IMI.

Dry period length and function of circulating immune cells

We are unaware of data pertaining to the immune status of cows given short or no dry periods before parturition. Research directed at understanding the status of the innate and acquired immune factors during the prepartum period for cows given short or no dry periods may provide important data concerning the interaction of DPL and immune function of dairy cows. Dairy cows experience many endocrine (Burvenich et al., 1999; Paape et al., 2002) and sudden metabolic changes during the periparturient period (Goff and Horst, 1997; Goff et al., 2002). Acute deficiencies of nutritional factors that are necessary for maintenance of the immune system occur when parturition approaches and this can last from a few days to several weeks (Goff and Horst, 1997). It seems logical that modifications of DPL may influence the cellular immune status of cows by altering the metabolic, hormonal/local or nutritional status at prepartum (Fig. 3).

Modulation of leucocyte function by metabolic factors

It appears that the prepartum metabolic profile is altered in cows with modified DPL. Low glucose, ketosis, fatty liver, and elevated non-esterified fatty acids (NEFA) are known to have negative effects on immune function in cattle (Targowski and Klucinski, 1983; Hoeben et al., 1997, 2000; Kaneene et al., 1997; Wentink et al., 1997; Sartorelli et al., 1999; Lacetera et al., 2001, 2005; Burvenich et al., 2007). Lower concentrations of NEFA periparturiently have been reported for cows with SDP or omitted dry periods (Andersen et al., 2005; Rastani et al., 2005; Pezeshki et al., 2007; Watters et al., 2008). Beta-hydroxy butyrate (BHBA) and glucose concentrations remain unchanged periparturiently between cows with standard and shortened or eliminated dry periods (Rastani et al., 2005; Pezeshki et al., 2007). However, lower concentrations of BHBA in early lactation and higher concentrations of glucose at peripartum have also been reported in CM cows (Andersen et al., 2005). We demonstrated that serum concentrations of triglycerides do not differ between SDP and control cows (Pezeshki et al., 2007). Concentrations of liver triglycerides and liver glycogen in CM and SDP cows do not differ from those of cows on standard dry periods at prepartum but may have lower liver triglycerides than controls during the subsequent lactation (Andersen et al., 2005; Rastani et al., 2005).

Lymphocyte function and number are reduced by a negative energy balance. Blood T-cell populations decrease as parturition approaches and reach a nadir at calving (Kimura et al., 1999a). Impaired lymphocyte function and response to mitogens around parturition have been demonstrated (Kehrli et al., 1989b; Saad et al., 1989; Lacetera et al., 2005). The CD4:CD8 T cells ratio in bovine blood varies depending on the lactation stage, and this ratio is 3:1

around calving (Hurley et al., 1990; Park et al., 1992; Shafer-Weaver et al., 1996; Lacey-VanKampen and Mallard, 1998). In general, lymphocyte and granulocyte function may be inhibited by increased levels of ketone bodies and/or NEFA, and by decreased levels of glucose and/or insulin (Kremer et al., 1993; Wentink et al., 1997; Sartorelli et al., 2000; Lacetera et al., 2002, 2004). Ketosis and fatty liver have also been associated with leucopenia (Reid et al., 1986; Franklin et al., 1991). This is because glucose provides the major energy supply for leucocytes (Weisdorf et al., 1982), whereas ketone bodies are highly diffusible and less metabolizable by leucocytes (Lavau et al., 1978; Lean et al., 1992). Body condition score reflects the energy status that may influence lymphocyte function in cows around calving (Lacetera et al., 2005). It has been shown that peripheral blood mononuclear cells from fat cows secrete less IFN-γ than those from thin cows or cows of medium condition 1 week before calving (Lacetera et al., 2004). Several mechanisms have been suggested to account for interference of fatty acids with lymphoid cell functions: perturbation of properties of cellular membranes, suppression of cytokine synthesis, formation of lipid peroxides and induction of necrosis or apoptosis (De Pablo and De Cienfuegos, 2000).

Important PMN functions, like migration and phagocytosis, are also impaired at parturition and may be attributed to negative energy status and endocrine changes (Kehrli et al., 1989b; Saad et al., 1989). The phagocytic capacity of macrophages is also decreased at parturition. Adhesion molecules CD62L (L-selectin) and CD11b/CD18 (Mac-1) are of vital importance for migration of blood PMN to the site of inflammation (Burton et al., 1995; Nagahata et al., 1995; Lee and Kehrli, 1998; Preisler et al., 2000). Decreased expression of L-selectin on circulatory PMN has been reported around parturition (Kimura et al., 1999b; Meglia et al., 2001; Monfardini et al., 2002). The expression of Mac-1 on bovine PMN was shown to be increased gradually as parturition approaches (Lee and Kehrli, 1998). These phenomena are responsible for the short lasting increase in circulatory PMN around parturition. The activity of myeloperoxidase by bovine PMN begins to decline from baseline approximately 2 weeks prepartum and reaches a minimum level during the first 7–10 days postpartum (Kimura et al., 1999b). Phagocytic activity of bovine PMN is reduced in parallel with decreased serum glucose concentrations (Newbould, 1973). Negative relationships between PMN functions and plasma concentrations of ketone bodies have previously been described for sheep, dairy cows and human (Hoeben et al., 1997, 2000; Sartorelli et al., 2000; Suriyasathaporn et al., 2000; Burvenich et al., 2007). The phagocytic activity of blood and milk macrophages is also decreased when cells are incubated *in vitro* with BHBA and acetoacetate (Klucinski et al., 1988).

Modulation of leucocyte function by hormonal factors

There is very little information about changes in systemic and local endocrine factors that are influenced by DPL in the peripartum period. Greater concentrations of serum oestradiol before calving were reported for cows with traditional dry periods than for cows with no dry period (Gumen et al., 2005). However, circulating progesterone was not altered by DPL in the same experiment (Gumen et al., 2005). Plasma insulin and ST concentrations during late pregnancy were not influenced by omission of the dry period, but greater concentrations of insulin were reported for CM cows during early stages of the next lactation (Andersen et al., 2005). We showed that serum concentration of insulin and IGF-I in early lactation were not influenced by shortening length of the dry period (Pezeshki et al., 2007).

Lymphocyte function and number are influenced by hormonal and local changes. Insulin may influence the activity of lymphocyte subpopulations by interfering with the expression of insulin-like growth factors (Kooijman et al., 1992). Corticosteroids are known to inhibit lymphocyte activation (Guidry et al., 1976) and high concentrations of serum cortisol at parturition may influence lymphocyte activity in cows (Ishikawa, 1987), as glucocorticoids are known to suppress immunity (Roth et al., 1982b; Lan et al., 1995). The previous literature has shown that $\delta\gamma$ T cells in the blood, IgM in mammary secretions and expression of major histocompatibility complex on mononuclear cells are all decreased by glucocorticoids (Nonnecke et al., 1997; Kehrli et al., 1999; Burton and Erskine, 2003). Regulatory roles of steroid hormones on maternal immune response during pregnancy have been proposed (Siiteri et al., 1977), and it has been demonstrated that progesterone inhibits lymphocyte activation *in vitro* (Mendelson et al., 1977; Wyle and Kent, 1977; Mori et al., 1997). Recombinant bovine ST (rbST) improves T-cell proliferation (Burton et al., 1991b), increases the proliferative responsiveness of peripheral blood lymphocytes (Burton et al., 1991b), enhances the number of circulating PMN (Burton et al., 1992) and augments the production of

reactive oxygen intermediates in the phagosome of neutrophils (Arkins et al., 1993).

Alterations in hormonal and local factors can also influence PMN function and number. Corticosteroids are released from the adrenal cortex in response to stress and can have significant effects on the circulation and functional capacities of immune cells (Roth and Flaming, 1990; Dhabhar et al., 1995; Dhabhar and McEwen, 1996). Neutrophilia is induced by corticosteroids, by an increasing output of PMN from the bone marrow and by increasing PMN demargination from the blood vessel wall (Roth et al., 1982b; Lee and Kehrli, 1998). PMN function is impaired and blood leukogram is altered by high concentrations of cortisol (Murray and Chenault, 1982; Roth et al., 1982a; Jayappa and Loken, 1983; Burton et al., 1995). Changes in progesterone, oestradiol, IGF-I and ST have the potential to modify PMN and lymphocyte functions (Moreira da Silva et al., 1997). Enhanced PMN function in response to rbST has been reported (Kelley, 1989; Burvenich et al., 1999). However, no effect of rbST on chemiluminescence, diapedesis and the expression of adhesion receptors of circulating PMN was reported in another study (Hoeben et al., 1999). IGF-I inhibits apoptosis of freshly isolated peripheral blood PMN, without altering the secretion of IL-8, IL-6 or TNF-α by these cells (Kooijman et al., 2002). A high level of progesterone negatively affects *in vitro* PMN oxidative burst activity (Moreira da Silva et al., 1997). Prepartum changes in bovine PMN functions have been linked to high 17β-oestradiol concentrations in blood plasma (Roth et al., 1982a, 1983; Burvenich et al., 2007). Conversely, the oxidative burst activity of PMN from ovariectomized cows was not changed significantly *in vitro* at both physiological and pharmacological levels of progesterone, 17β-oestradiol and oestrone (Winters et al., 2003). The number of viable PMN was decreased after 17β-oestradiol treatment, but no effect of progesterone was found (Lamote et al., 2004a). 17β-oestradiol treatment was shown to inhibit the proliferation of granulocyte progenitor cells *in vitro* (Van Merris et al., 2004). In general, the influence of sex steroids on PMN function and number is very complex and their mechanism of action is not completely understood. Furthermore, some discrepancies have been found between *in vitro* and *in vivo* studies. Information on the interaction between sex steroids and bovine PMN is also scarce (Burvenich et al., 2003). It has been hypothesized that 17β-oestradiol may contribute to the induction of a compromised PMN function around parturition (Burvenich et al., 2007). Recently, we detected oestrogen receptors on bovine blood PMN at both protein and mRNA level (Lamote et al., 2006, 2007). Glucocorticoids prevent PMN margination by reducing the expression of L-selectin and CD18 on PMN (Burton et al., 1995). In conclusion, PMN function in dairy cows is most likely affected by multitude of endocrine factors.

Modulation of leucocyte function by nutritional factors

The function and number of PMN and lymphocytes are also influenced by changes in nutritional factors. Chronic decreases in retinol, α-tocopherol, Zinc and selenium in plasma are associated with severe health problems and loss of immune function (Herdt and Stowe, 1991; Michal et al., 1994; Smith et al., 1997). These micronutrients are cellular antioxidants, preventing peroxidative damage in cell membranes (vitamins) or in the cytoplasm (trace elements), and are essential for proper functioning of the immune system (Miller et al., 1993; Weiss, 2002). Phagocytosis and intracellular killing of some mastitis pathogens by PMN is suppressed by selenium deficiency (Gyang et al., 1984; Smith et al., 1984, 1997; Grasso, 1987). High concentrations of 1,25-$(OH)_2$ D are known to impair PMN function and lymphocyte blastogenesis (Lemire et al., 1985; Reichel et al., 1987). Adhesion of circulating leucocytes to the vascular endothelium is critical for effective host inflammatory and immune responses (Paape et al., 1991). Calcium and magnesium are required for proper expression of the CDllb epitope on PMN (Leino and Sorvajarvi, 1992; Leino and Paape, 1993). The CDllb epitope together with CD18 forms the complement receptor 3 (CR3). This receptor is essential for adherence of PMN to endothelium and their subsequent recruitment to sites of infection (Kehrli and Shuster, 1994). The CR3 receptor also promotes binding of both unopsonized bacteria and C3bi opsonized bacteria. Moreover, phagocytosis of opsonized and unopsonized *Escherichia coli* is calcium- and magnesium-dependent (Dosogne et al., 1998). A lowered bactericidal activity of blood leucocytes in cattle and sheep may be caused by copper deficiency (Jones and Suttle, 1981; Xin et al., 1991), probably because of decreased superoxide dismutase activity of PMN caused by copper deficiency (Xin et al., 1991). Function of lymphocytes and PMN also are impaired during retinol deficiency (Ongsakul et al., 1985; Tjoelker et al., 1988). *In vitro* phagocytosis and intracellular killing of live *Staphylococcus aureus* by bovine milk PMN are stimulated by retinol and retinoic acid (Tjoelker et al., 1990). β-carotene increased the killing ability of blood and milk phagocytes (Daniel et al., 1991b) as well as

in vitro proliferation of peripheral blood lymphocytes (Daniel et al., 1991a) from peripartum dairy cows. An association has been found between plasma vitamin E concentration and PMN function and IMI rate (Hogan et al., 1989, 1992; Kehrli et al., 1989a; Weiss et al., 1990). Deficiency of α-tocopherol reduces phagocytic cell function (Boxer et al., 1979; Boxer, 1986; Hogan et al., 1990). Vitamin E and glutathione peroxidase both are cellular antioxidants at membrane and cytosol, respectively, protecting phagocytic cells and surrounding tissues from the destructive action of toxic oxygen molecules (Putnam and Comben, 1987; Hogan et al., 1990, 1993). 1,25-$(OH)_2$ D induces less production of IFN-γ and IL-2, and more IL-4, IL-5, and IL-10 by lymphocytes (Daynes et al., 1996). Beneficial effects of vitamin A and β-carotene on lymphocyte function have also been noted (Chew, 1987). We demonstrated that all-*trans*- and 9-*cis*-retinoic acid stimulate the growth of granulocytes colonies (Van Merris et al., 2004). In general, dietary deficiencies have been associated with increased prevalence of mastitis. For example, when concentration of vitamin E is the lowest in dairy cows, the occurrence of IMI is the highest and function of PMN is depressed. Function of PMN and lymphocytes is influenced negatively as a result of deficiency of nutritional factors. To our knowledge, there is no information about alteration in nutritional factors periparturiently in cows with modified DPL.

Conclusion

The results of most recent studies on DPL support past research, indicating that 8-week DPL is still required for today's high-producing cows for maximal milk production in the subsequent lactation. Omitting the dry period completely in dairy cows causes considerable milk production loss in the next lactation. This is most likely attributed to reduced cell turnover and replacement of senescent MEC and progenitor cells during late gestation in these animals, so that senescent MEC have less secretory ability and proliferative capability in the following lactation. However, there is some evidence that administration of bovine ST during lactation may reduce the necessity for an 8-week dry period. While mammary involution has previously been studied in CM cows and cows with traditional dry periods, there is no information about mammary involution and cell kinetics in cows with SDP, and more research in this area would be beneficial. Milk yield depression in cows with SDP may be explained by inhibited early events of involution, as a result of enhanced inhibitory effects of pregnancy on MEC loss and lower milk production at drying off. Shortening or eliminating the dry period, however, was shown to improve energy status of periparturient cows by reducing the frequency of dietary changes and facilitating delivery of a high-energy diet at the end of gestation. Thus, it appears that milk loss in the subsequent lactation in cows with SDP or omitted dry periods is mainly a result of altered mammary involution and cell proliferation, rather than systemic effects of metabolic factors.

Very little is known about the immune status of cows with modified DPL. Particularly, the bovine mammary gland defence in cows with modified DPL is an area in need of further research. Shortening the dry period may improve the ability of the immune system to combat IMI, which is typically compromised at dry-off. We predict this to be a consequence of minimizing the accumulation of milk or its components in the mammary gland, because this accumulation interferes with efficacy of phagocytic components of the immune system. Milking cows continuously may also strengthen the immune system by not only removing milk accumulation effects, but by increasing the flushing and teat end disinfection during late gestation. Modifying the length of the dry period may influence the rate of new IMI occurrence during the dry period, by changing the composition of mammary secretions. Additionally, the immune system of cows with altered DPL may be influenced periparturiently by the impact on metabolic, hormonal, local and nutritional factors. We conclude that there is a need to integrate mammary gland biology and defence mechanisms in studies dealing with modified DPLs.

In this review, we have presented evidences for a negative association between the level of milk production (and milk accumulation) at dry-off, and cure rate of IMIs. Reduced milk accumulation at drying off lessens the immunodeficiency over this period by hastening the process of early involution. During the mid dry period there is no milk secretion, and inflammation is not necessary for the defence of the gland against invasion of pathogens. Consequently, there is less damage to cells. In many cows, milk production decreases gradually as lactation proceeds and calving approaches. However, in some cows, milk production does not show such a striking gradual decrease and milk production remains high until the end of lactation. Specifically in these cows, shortening the DPL permits having a prolonged lactation and consequently minimized milk

production at dry-off. Immune deficiency might be reduced in SDP cows because of low milk production at dry-off. In addition to management of the DPL, there could be other options to achieve this goal. A small molecular weight glycoprotein, feedback inhibitor of lactation (FIL), has been proposed to be involved in the reduction of milk synthesis and functional differentiation of secretory cells at milk stasis (Knight et al., 1998). Identification and characterization of new molecules may open perspectives for the development of new drugs. Proteomics and peptidomics should be of great help in identifying the candidate proteins and peptides involved in local control of milk secretion. These molecules and their possible interactions with FIL may provide important keys to manipulating early involution and mammary defence. Finally, modification of the length of calving interval could be another management option to manipulate the involution process after milk cessation. Although, economically desired calving interval is now around 1 year, lengthening the calving interval might have a modulatory effect on mammary defence against infections in terms of relatively reduced milk production and lowered immunodeficiency over drying off in these cows. There is, however, no direct evidence to change the management of the dry period in terms of mammary defence.

References

Accorsi, P. A.; Pacioni, B.; Pezzi, C.; Forni, M.; Flint, D. J.; Seren, E., 2002: Role of prolactin, growth hormone and insulin-like growth factor 1 in mammary gland involution in the dairy cow. *Journal of Dairy Science* **85**, 507–513.

Akers, R. M.; McFadden, T. B.; Beal, W. E.; Guidry, A. J.; Farrell, H. M., 1986: Radioimmunoassay for measurement of bovine α-lactalbumin in serum, milk, and tissue culture media. *Journal of Dairy Research* **53**, 419–429.

Andersen, J. B.; Madsen, T. G.; Larsen, T.; Ingvartsen, K. L.; Nielsen, M. O., 2005: The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. *Journal of Dairy Science* **88**, 3530–3541.

Annen, E. L.; Collier, R. J., 2005: Modified dry periods in dairy cattle: implications for milk yield and the transition period. *Southwest Nutrition and Management Conference*, University of Arizona, Arizona, pp. 209–224. Available at: http://cals.arizona.edu/ans/swnmc/.

Annen, E. L.; Collier, R. J.; McGuire, M. A.; Vicini, J. L.; Ballam, J. M.; Lormore, M. J., 2004a: Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *Journal of Dairy Science* **87**, 3746–3761.

Annen, E. L.; Collier, R. J.; McGuire, M. A.; Vicini, J. L., 2004b: Effects of dry period length on milk yield and mammary epithelial cells. *Journal of Dairy Science* **87**, E66–E76.

Annen, E. L.; Fitzgerald, A. C.; Gentry, P. C.; McGuire, M. A.; Capuco, A. V.; Baumgard, L. H.; Collier, R. J., 2007: Effect of continuous milking and bST supplementation on mammary epithelial cell turnover. *Journal of Dairy Science* **90**, 165–183.

Arkins, S.; Dantzer, R.; Kelley, K. W., 1993: Somatolactogens, somatomedins, and immunity. *Journal of Dairy Science* **76**, 2437–2450.

Arnold, P. T. Dix; Becker, R. B., 1936: Influence of preceding dry period and of mineral supplementation on lactation. *Journal of Dairy Science* **19**, 257–266.

Athie, F.; Bachman, K. C.; Head, H. H.; Hayen, M. J.; Wilcox, C. J., 1996: Estrogen administered at final milk removal accelerates involution of bovine mammary gland. *Journal of Dairy Science* **79**, 220–226.

Bachman, K. C., 2002: Milk production of dairy cows treated with estrogen at the onset of a short dry period. *Journal of Dairy Science* **85**, 797–803.

Bachman, K. C.; Schairer, M. L., 2003: Invited review: bovine studies on optimal lengths of dry periods. *Journal of Dairy Science* **86**, 3027–3037.

Boxer, L. A., 1986: Regulation of phagocyte function by α-tocopherol. *Proceedings of the Nutrition Society* **45**, 333–344.

Boxer, L. A.; Oliver, J. M.; Spielberg, S. P.; Allen, J. M.; Schulman, J. D., 1979: Protection of granulocytes by vitamin E in glutathione synthetase deficiency. *New England Journal of Medicine* **301**, 901–905.

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.

Brandon, M. R.; Lascelles, A. K., 1975: The effect of pre-partum milking on the transfer of immunoglobulin into mammary secretion of cows. *Australian Journal of Experimental Biology and Medical Science* **53**, 197–204.

Breau, W. C.; Oliver, S. P., 1985: Accelerated bovine mammary involution induced by infusion of concanavalin A or phytohemagglutinin. *American Journal of Veterinary Research* **46**, 816–820.

Breau, W. C.; Oliver, S. P., 1986: Growth inhibition of eavironmeatal mastitis pathogens during physiologic transitions of the bovine mammary gland. *American Journal of Veterinary Research* **47**, 218–222.

Burton, J. L.; Erskine, R. J., 2003: Immunity and mastitis. Some new ideas for an old disease. *Veterinary Clinics of North America: Food Animal Practice* **19**, 1–45.

Burton, J. L.; McBride, B. W.; Kennedy, B. W.; Burton, J. H.; Elsasser, T. H.; Woodward, B., 1991a: Serum immunoglobulin profiles of dairy cows chronically treated with recombinant bovine somatotropin. *Journal of Dairy Science* **74**, 1589–1598.

Burton, J. L.; McBride, B. W.; Kennedy, B. W.; Burton, J. H.; Elsasser, T. H.; Woodward, B., 1991b: Influence of exogenous bovine somatotropin on the responsiveness of peripheral blood lymphocytes to mitogen. *Journal of Dairy Science* **74**, 916–928.

Burton, J. L.; McBride, B. W.; Kennedy, B. W.; Burton, J. H.; Elsasser, T. H.; Woodward, B., 1992: Hematological profiles in dairy cows treated with recombinant bovine somatotropin. *Journal of Animal Science* **70**, 1488–1495.

Burton, J. L.; Kehrli, M. E. J.; Kapil, S.; Horst, R. L., 1995: Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. *Journal of Leukocyte Biology* **57**, 317–325.

Burvenich, C.; Peeters, G., 1980: Effect of intramammary infusion of colchicine on mammary blood flow in lactating goats. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **44**, 211–217.

Burvenich, C.; Paape, M. J.; Hoeben, D.; Dosogne, H.; Massart-Leen, A. M.; Blum, J., 1999: Modulation of the inflammatory reaction and neutrophil defense of the bovine lactating mammary gland by growth hormone. *Domestic Animals Endocrinology* **17**, 149–159.

Burvenich, C.; Detilleux, J.; Paape, M.; Massart-Leen, A., 2000: Physiological and genetic factors that influence the cows resistance to mastitis, especially during early lactation. In: A. Zecconi (ed.), *Proceedings of the IDF Symposium on Immune Ruminant Mammary Gland*, Stresa, Italy, pp. 9–20.

Burvenich, C.; Van Merris, V.; Mehrzad, J.; Diez-Fraile, A.; Duchateau, L., 2003: Severity of *E.coli* mastitis is mainly determined by cow factors. *Veterinary Research* **34**, 521–564.

Burvenich, C.; Bannerman, D. D.; Lippolis, J. D.; Peelman, L.; Nonnecke, B. J.; Kehrli, M. E. J.; Paape, M. J., 2007: Cumulative physiological events influence the inflammatory response of the bovine udder to *Escherichia coli* infections during the transition period. *Journal of Dairy Science* **90**, E39–E54.

Bushe, T.; Oliver, S. P., 1987: Natural protective factors in bovine mammary secretion following different methods of milk cessation. *Journal of Dairy Science* **70**, 696–704.

Butler, J. E., 1983: Bovine immunoglobulins: an augmented review. *Veterinary Immunology and Immunopathology* **4**, 43–152.

Capuco, A. V.; Akers, R. M., 1990: Thymidine incorporation by lactating mammary epithelium during compensatory mammary growth in beef cattle. *Journal of Dairy Science* **73**, 3094–3103.

Capuco, A. V.; Akers, R. M., 1999: Mammary involution in dairy animals. *Journal of Mammary Gland Biology and Neoplasia* **4**, 137–144.

Capuco, A. V.; Akers, R. M.; Smith, J. J., 1997: Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. *Journal of Dairy Science* **80**, 477–487.

Capuco, A. V.; Li, M.; Long, E.; Ren, S.; Hrusk, K. S.; Schorr, K.; Furth, P. A., 2002: Pregnancy retards mammary involution: effects on apoptosis and proliferation of the mammary epithelium after forced weaning of mice. *Biology of Reproduction* **66**, 1471–1476.

Capuco, A. V.; Ellis, S. E.; Hale, S. A.; Long, E.; Erdman, R. A.; Zhao, X.; Paape, M. J., 2003: Lactation persistency: insights from mammary cell proliferation studies. *Journal of Animal Science* **81**, 18–31.

Capuco, A. V.; Annen, E.; Fitzgerald, A. C.; Ellis, S. E.; Collier, R. J., 2006: Mammary cell turnover: relevance to lactation persistency and dry period management. In: K. Sejrsen, T. Hvelplund, M. O. Nielsen (eds), *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 363–388.

Chew, B. P., 1987: Vitamin A and β-carotene on host defense. *Journal of Dairy Science* **70**, 2732–2743.

Church, G. T.; Fox, L. K.; Gaskins, C. T.; Hancock, D. D.; Gay, J. M., 2008: The effect of a shortened dry period on intramammary infections during the subsequent lactation. *Journal of Dairy Science* **91**, 4219–4225.

Clarkson, R. W.; Wayland, M. T.; Lee, J.; Freeman, T.; Watson, C. J., 2004: Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. *Breast Cancer Research* **6**, R92–R109.

Collier, R. J.; McGrath, M. F.; Byatt, J. C.; Zurfluh, L. L., 1993: Regulation of bovine mammary growth by peptide hormones: involvement of receptors, growth factors and binding proteins. *Livestock Production Science* **35**, 21–33.

Comalli, M. P.; Eberhart, R. J.; Oriel, L. C.; Rothea-bacher, H., 1984: Changes in the microscopic anatomy of the bovine teat canal during mammary involution. *American Journal of Veterinary Research* **45**, 2236–2242.

Coppock, C. E.; Everett, R. W.; Natzke, R. P.; Ainslie, H. R., 1974: Effect of dry period length on Holstein milk production and selected disorders at parturition. *Journal of Dairy Science* **57**, 712–717.

Cousins, C. L.; Higgs, T. M.; Jackson, E. R.; Neave, F. K.; Dodd, F. H., 1980: Susceptibility of the bovine udder to bacterial infection in the dry period. *Journal of Dairy Science* **47**, 11–18.

Craven, N.; Williams, M. R., 1985: Defenses of the bovine mammary gland against infection and prospects for their enhancement. *Veterinary Immunology and Immunopathology* **10**, 71–127.

Daniel, L. R.; Chew, B. P.; Tanaka, T. S.; Tjcelker, L. W., 1991a: In vitro effects of β-carotene and vitamin A on peripartum bovine peripheral blood mononuclear cell proliferation. *Journal of Dairy Science* **74**, 911–915.

Daniel, L. R.; Chew, B. P.; Tanaka, T. S.; Tjoelker, L. W., 1991b: β-Carotene and vitamin A effects on bovine phagocyte function in vitro during the periparturn period. *Journal of Dairy Science* **74**, 124–131.

Daynes, R. A.; Enioutina, E. Y.; Butler, S.; Mu, H. H.; McGee, Z. A.; Araneo, B. A., 1996: Induction of common mucosal immunity by hormonally mmunomodulated peripheral immunization. *Infection and Immunity* **64**, 1100–1109.

De Pablo, M. A.; De Cienfuegos, A., 2000: Modulatory effects of dietary lipids on immune system functions. *Immunology and Cell Biology* **78**, 31–39.

De Spiegeleer, B.; Vergote, V.; Pezeshki, A.; Peremans, K.; Burvenich, C., 2008: Impurity profiling quality control testing of synthetic peptides using liquid. chromatography-photodiode array-fluorescence and liquid chromatographyelectrospray ionization-mass spectrometry: the obestatin case. *Analitical Biochemistry* **376**, 229–234.

Detilleux, J. C.; Koehler, K. J.; Freeman, A. E.; Kehrli, M. E. J.; Kelley, D. H., 1994: Immunological parameters of periparturient Holstein cattle: genetic variation. *Journal of Dairy Science* **77**, 2640–2650.

Dhabhar, F. S.; McEwen, B. S., 1996: Stress-induced enhancement of antigen-specific cell-mediated immunity. *Journal of Immunology* **156**, 2608–2615.

Dhabhar, F. S.; Miller, A. H.; McEwen, B. S.; Spencer, R. L., 1995: Effects of stress on immune cell distribution: dynamics and hormonal mechanisms. *Journal of Immunology* **154**, 5511–5527.

Dias, F. M.; Allaire, F. R., 1982: Dry period to maximize milk production over two consecutive lactations. *Journal of Dairy Science* **65**, 136–145.

Dickerson, G. E.; Chapman, A. B., 1939: The effect of age and dry period on production at different levels of producing ability. *Journal of Animal Science* **13**, 73–76.

Dirksen, G. U.; Liebich, H. G.; Mayer, E., 1985: Adaptive changes of the ruminal mucosa and their functional and clinical significance. *Bovine Practice* **20**, 116–120.

Dosogne, H.; Burvenich, C.; Paape, M. J., 1998: Effect of extracellular ionic calcium and magnesium on opsonic and non-opsonic phagocytosis of *Escherichia coli* bovine blood polymorphonuclear leucocytes. *Comparative Haematology International* **8**, 82–86.

Elfstrand, L.; Lindmark-mansson, H.; Paulsson, M.; Nyberg, L.; Akesson, B., 2002: Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal* **12**, 879–887.

Feng, Z.; Marti, A.; Jehn, B.; Altermatt, H. J.; Chicaiza, G.; Jaggi, R., 1995: Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. *Journal of Cell Biology* **131**, 1095–1103.

Fitzgerald, A. C.; Annen-Dawson, E. L.; Baumgard, L. H.; Collier, R. J., 2007: Evaluation of continuous lactation and increased milking frequency on milk production and mammary cell turnover in primiparous Holstein cows. *Journal of Dairy Science* **90**, 5483–5489.

Fleet, I. R.; Goode, J. H.; Hamon, M. H.; Laurie, M. S.; Linzell, J. L.; Peaker, M., 1975: Secretory activity of the goat mammary glands during pregnancy and the onset of lactation. *Journal of Physiology* **251**, 763–773.

Franklin, S. T.; Young, J. W.; Nonnecke, J., 1991: Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. *Journal of Dairy Science* **74**, 2507–2514.

Funk, D. A.; Freeman, A. E.; Berger, P. J., 1987: Effects of previous days open, previous days dry, and present days open on lactation yield. *Journal of Dairy Science* **70**, 2366–2373.

Gallo, L.; Contiero, B.; De Marchi, M.; Carnier, P.; Cassandro, M.; Bittante, G., 2008: Retrospective analysis of dry period length in Italian Holstein cows. *Italian Journal of Animal Science* **7**, 65–76.

Goff, J. P.; Horst, R. L., 1997: Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science* **80**, 1260–1268.

Goff, J. P.; Kimura, K.; Horst, R. L., 2002: Effect of mastectomy on milk fever, energy, and vitamins A, E, and β-Carotene status at parturition. *Journal of Dairy Science* **85**, 1427–1436.

Goodman, R. E.; Schanbacher, F. L., 1991: Bovine lactoferrin mRNA: sequence, analysis and expression in the mammary gland. *Biochemical and Biophysical Research Communications* **180**, 75–84.

Grasso, P. J., 1987: Phagocytosis, bactericidal activity, and oxidative metabolism of mammary neutrophils from selenium-adequate and selenium-deficient dairy cows. MS thesis, The Pennsylvania State University, University Park, PA, USA.

Griffith, E.; Humphrey, J., 2004: Bacteriostatic effect of human milk and bovine colostrum on *Escherichia coli*. The importance of bicarbonate. *Infection and Immunity* **15**, 396–401.

Grummer, R. R.; Rastani, R. R., 2004: Why re-evaluate dry period length. *Journal of Dairy Science* **87**, E77–E85.

Grummer, R. R.; Rastani, R. R., 2005: Strategies for shortening the dry period. *Proceedings of the 7th Western Dairy Management Conference*, Reno, Nevada, pp, 129–140. Available at: http://www.wdmc.org/2005/13Grummera.pdf

Guidry, A. J.; Paape, M. J.; Pearson, R. E., 1976: Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. *American Journal of Veterinary Research* **37**, 1195–1200.

Gulay, M. S.; Hayen, M. J.; Bachman, K. C.; Belloso, T.; Liboni, M.; Head, H. H., 2003: Milk production and feed intake of Holstein cows given short (30-d) or normal (60-d) dry periods. *Journal of Dairy Science* **86**, 2030–2038.

Gulay, M. S.; Hayen, M. J.; Liboni, M.; Belloso, T. I.; Wilcox, C. J.; Head, H. H., 2004: Low doses of bovine somatotropin during transition period and early lactation improves milk yield, efficiency of production, and other physiological responses of Holstein cows. *Journal of Dairy Science* **87**, 948–960.

Gulay, M. S.; Hayen, M. J.; Head, H. H.; Wilcox, C. J.; Bachman, K. C., 2005: Milk production from Holstein half udders after concurrent thirty- and seventy-day dry periods. *Journal of Dairy Science* **88**, 3953–3962.

Gumen, A.; Rastani, R. R.; Grummer, R. R.; Wiltbank, M. C., 2005: Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *Journal of Dairy Science* **88**, 2401–2411.

Guy, M. A.; McFadden, T. B.; Cockrell, D. C.; Besser, T. E., 1994: Regulation of colostrum formation in beef and dairy cows. *Journal of Dairy Science* **77**, 3002–3007.

Gyang, E. A.; Stevens, J. B.; Olson, W. G.; Tsitsamis, S. D.; Usenik, E. A., 1984: Effects of selenium-vitamin E injection on bovine polymorphonucleated leukocytes phagocytosis and killing Staphylococcus aureus. *American Journal of Veterinary Research* **45**, 175–177.

Harmon, R. J., 1994: Physiology of mastitis and factors affecting somatic cell counts. *Journal of Dairy Science* **77**, 2103–2112.

Herdt, T. H.; Stowe, H. D., 1991: Fat-soluble vitamin nutrition for dairy cattle. *Veterinary Clinics of North America: Food Animal Practice* **7**, 391–415.

Hirsch, H. P., 1987: Zellzahl und Zellbild des eutersekrets von Kuhen vor dem trockenstellen und zubeginn der trockenperiode. *Archives of Experimental Veterinary Medicine* **41**, 1–6.

Hoeben, D.; Heyneman, R.; Burvenich, C., 1997: Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. *Veterinary Immunology and Immunopathology* **58**, 165–170.

Hoeben, D.; Burvenich, C.; Eppard, P. J.; Byatt, J. C.; Hard, D. L., 1999: Effect of bovine somatotropin on neutrophil functions and clinical symptoms during *Streptococcus uberis* mastitis. *Journal of Dairy Science* **82**, 1465–1481.

Hoeben, D.; Monfardini, E.; Opsomer, G.; Burvenich, C.; Dosogne, H.; De Kruif, A.; Beckers, J. F., 2000: Chemiluminescence of bovine polymorphonuclear leucocytes during the periparturient period and relation with metabolic markers and bovine pregnancy-associated glycoprotein. *Journal of Dairy Science* **67**, 249–259.

Hogan, J. S.; Smith, K. L., 2003: Coliform mastitis. *Veterinary Research* **34**, 507–519.

Hogan, J. S.; Smith, K. L.; Hoblet, K. H.; Schoenberger, P. S.; Todhunter, D. A.; Hueston, W. D.; Pritchard, D. E.; Bowman, G. L.; Heider, L. E.; Brockat, B. L.; Conrad, H. R., 1989: Field survey of clinical mastitis in low somatic cell count herds. *Journal of Dairy Science* **72**, 1547–1556.

Hogan, J. S.; Smith, K. L.; Weiss, W. P.; Todhunter, D. A.; Shockey, W. L., 1990: Relationships among vitamin E, selenium, and bovine blood neutrophils. *Journal of Dairy Science* **73**, 2372–2378.

Hogan, J. S.; Weiss, W. P.; Todhunter, D. A.; Smith, K. L.; Schoenberger, P. S., 1992: Bovine neutrophil responses to parenteral vitamin E. *Journal of Dairy Science* **75**, 399–405.

Hogan, J. S.; Weiss, W. P.; Smith, K. L., 1993: Role of vitamin E and selenium in host defense against mastitis. *Journal of Dairy Science* **76**, 2795–2803.

Holst, B. D.; Hurley, W. L.; Nelson, D. R., 1987: Involution of the bovine mammary gland: histological and ultrastructural changes. *Journal of Dairy Science* **70**, 935–944.

Huguet, E. L.; Smith, K.; Bicknell, R.; Harris, A. L., 1995: Regulation of Wnt5a expression in human mammary epithelial cells by cell shape, confluence and hepatocyte growth factor. *The Journal of Biological Chemistry* **270**, 12851–12856.

Hurley, W. L., 1987: Mammary function during the nonlactating period: enzyme, lactose, protein concentrations, and pH of mammary secretions. *Journal of Dairy Science* **70**, 20–28.

Hurley, W. L., 1989: Mammary gland function during involution. *Journal of Dairy Science* **72**, 1637–1646.

Hurley, W. L.; Rejman, J. J., 1986: β-lactoglobulin and α-lactalbumin in mammary secretions during the dry period: parallelism of concentration changes. *Journal of Dairy Science* **69**, 1642–1647.

Hurley, D. J.; Kensinger, M. H.; Mastro, A. M.; Wilson, R. A., 1990: An evaluation of the mononuclear cells derived from bovine mammary gland dry secretions using leukocyte antigen specific monoclonal antibodies, light scattering properties and non-specific esterase staining. *Veterinary Immunology and Immunopathology* **25**, 177–193.

Huxley, J. N.; Green, M. J.; Green, L. E.; Bradley, A. J., 2002: Evaluation of the efficacy of an internal teat sealer during the dry period. *Journal of Dairy Science* **85**, 551–561.

Ishikawa, H., 1987: Observation of lymphocyte function in perinatal cows and neonatal calves. *Japanese Journal of Veterinary Science* **49**, 469–475.

Jayappa, H. G.; Loken, K. I., 1983: Effect ofantimicrobial agents and corticosteroids on bovine polymorphonuclear leukocyte chemotaxis. *American Journal of Veterinary Research* **44**, 2155–2159.

Jensen, D. L.; Eberhart, R. J., 1981: Total and differential cell counts in secretions of the nonlactating bovine mammary gland. *American Journal of Veterinary Research* **42**, 743–747.

Johnson, K. A.; Johnson, D. E., 1995: Methane emissions from cattle. *Journal of Animal Science* **73**, 2483–2492.

Jones, D. G.; Suttle, N. F., 1981: Some effects of copper deficiency on leucocyte function in sheep and cattle. *Research in Veterinary Science* **31**, 151–156.

Kaneene, J. B.; Miller, R. A.; Herdt, T. H.; Gardiner, J. C., 1997: The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Preventive Veterinary Medicine* **31**, 59–72.

Kehrli, J. M. E.; Shuster, D. E., 1994: Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *Journal of Dairy Science* **77**, 619–627.

Kehrli, J. M. E.; Nonnecke, B. J.; Roth, J. A., 1989a: Alterations in bovine neutrophil function during the peripartum period. *American Journal of Veterinary Research* **50**, 207–214.

Kehrli, J. M. E.; Nonnecke, B. J.; Roth, J. A., 1989b: Alterations in bovine peripheral blood lymphocyte function during the peripartum period. *American Journal of Veterinary Research* **50**, 215–220.

Kehrli, J. M. E.; Burton, J. L.; Nonnecke, B. J.; Lee, E. K., 1999: Effects of stress on leukocyte trafficking and immune responses: implications for vaccination. *Advanced Veterinary Medicine* **41**, 61–81.

Kelley, K. W., 1989: Growth hormone, lymphocytes and macrophages. *Biochemical Pharmacology* **5**, 705–713.

Kimura, K.; Goff, J. P.; Kehrli, J. M. E.; Harp, J. A., 1999a: Phenotype analysis of peripheral blood mononuclear cells in periparturient dairy cows. *Journal of Dairy Science* **82**, 315–319.

Kimura, K.; Goff, J. P.; Kehrli, J. M. E., 1999b: Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. *Journal of Dairy Science* **82**, 2385–2392.

Kishimoto, T. K.; Jutila, M. A.; Berg, E. L.; Butcher, E. C., 1989: Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science* **245**, 1238–1241.

Klucinski, W.; Degorski, A.; Miernik-Degorska, E.; Targowski, S.; Winnicka, A., 1988: Effect of ketone bodies on the phagocytic activity of bovine milk macrophages and polymorphonuclear leukocytes. *Journal of Veterinary Medicine A* **35**, 632–639.

Knight, C. H., 1998: Extended Lactation. Hannah Research Institute Year Book. The Hannah research, Ayr, pp. 30–39.

Knight, C. H.; Peaker, M.; Wilde, C. J., 1998: Local control of mammary development and function. *Reviews of Reproduction* **3**, 104–112.

Kooijman, R.; Willems, M.; De Haas, C. J. C.; Rijkers, G. T.; Schuurmans, A. L. G.; Van Buul- Offers, S. C.; Heijnen, C. J.; Zegers, B. J. M., 1992: Expression of type 1 insulin-like growth factor receptors on human peripheral blood mononuclear cells. *Endocrinology* **131**, 2244–2250.

Kooijman, R.; Coppens, A.; Hooghe-Peters, E., 2002: IGF-I inhibits spontaneous apoptosis in human granulocytes. *Endocrinology* **143**, 1206–1212.

Kremer, W. D. J.; Noordhuizen-Stassen, E. N.; Grommers, F. J.; Schukken, Y. H.; Heeringa, R.; Brand, A.; Butvenich, C., 1993: Severity of experimental *Escherichia coli* mastitis in ketonaemic and nonketonaemic dairy cows. *Journal of Dairy Science* **76**, 3428–3436.

Kuhn, M. T.; Hutchison, J. L., 2005: Methodology for the estimation of days dry effects. *Journal of Dairy Science* **88**, 1499–1508.

Kuhn, M. T.; Hutchison, J. L.; Norman, H. D., 2005: Minimum days dry to maximize milk yield in subsequent lactation. *Animal Research* **54**, 351–367.

Kuhn, M. T.; Hutchison, J. L.; Norman, H. D., 2006: Dry period length to maximize production across adjacent lactations and lifetime production. *Journal of Dairy Science* **89**, 1713–1722.

Kuhn, M. T.; Hutchison, J. L.; Norman, H. D., 2007: Dry period length in US Jerseys: characterization and effects on performance. *Journal of Dairy Science* **90**, 2069–2081.

Lacetera, N.; Bernabucci, U.; Ronchi, B.; Nardone, A., 2001: Effects of subclinical pregnancy toxemia on immune responses in sheep. *American Journal of Veterinary Research* **62**, 1020–1024.

Lacetera, N.; Franci, O.; Scalia, D.; Bernabucci, U.; Ronchi, B.; Nardone, A., 2002: Effects of nonesterified fatty acids and β-hydroxybutyrate on functions of mononuclear cells obtained from ewes. *American Journal of Veterinary Research* **63**, 414–418.

Lacetera, N.; Scalia, D.; Franci, O.; Bernabucci, U.; Ronchi, B.; Nardone, A., 2004: Effects of nonesterified fatty acids on lymphocyte function in dairy heifers. *Journal of Dairy Science* **87**, 1012–1014.

Lacetera, N.; Scalia, D.; Bernabucci, U.; Ronchi, B.; Pirazzi, D.; Nardone, A., 2005: Lymphocyte functions in overconditioned cows around parturition. *Journal of Dairy Science* **88**, 2010–2016.

Lacey-VanKampen, C.; Mallard, B. A., 1998: Effects of peripartum stress and disease on bovine lymphocyte

subsets. *Veterinary Immunology and Immunopathology* **59**, 79–92.

Lamote, I.; Meyer, E.; Duchateau, L.; Burvenich, C., 2004a: Influence of 17β-estradiol, progesterone, and dexamethasone on diapedesis and viability of bovine blood polymorphonuclear leukocytes. *Journal of Dairy Science* **87**, 3340–3349.

Lamote, I.; Meyer, E.; Massart-Leen, A. M.; Burvenich, C., 2004b: Review: sex steroids and growth factors in the regulation of mammary gland proliferation, differentiation, and involution. *Steroids* **69**, 145–159.

Lamote, I.; Meyer, E.; De Ketelaere, A.; Duchateau, L.; Burvenich, C., 2006: Expression of the estrogen receptor in blood neutrophils of dairy cows during the periparturient period. *Theriogenology* **65**, 1082–1098.

Lamote, I.; Demeyere, K.; Notebaert, S.; Burvenich, C.; Meyer, E., 2007: Flow cytometric assessment of estrogen receptor β expression in bovine blood neutrophils. *Journal of Immunological Methods* **323**, 88–92.

Lan, H. C.; Reddy, P. G.; Chambers, M. A.; Walker, G.; Srivastava, K. K.; Ferguson, J. A., 1995: Effect of stress on interleukin-2 receptor expression by bovine mononuclear leukocytes. *Veterinary Immunology and Immunopathology* **49**, 241–249.

Lascelles, A. K., 1979: The immune system of the ruminant mammary gland and its role in the control of mastitis. *Journal of Dairy Science* **62**, 154–160.

Lavau, M.; Fornari, V.; Hashim, S. A., 1978: Keystone metabolism in brain slices from rats with diet induced hyperketonemia. *Journal of Nutrition* **108**, 621–629.

Lean, I. J.; Bruss, M. L.; Baldwin, R. L.; Troutt, H. F., 1992: Bovine ketosis: a review II. Biochemistry and prevention. *The Veterinary Bulletin* **61**, 1–14.

Lee, E. K.; Kehrli, M., 1998: Expression of adhesion molecules on neutrophils of periparturient cows and neonatal calves. *American Journal of Veterinary Research* **59**, 37–43.

Leino, L.; Paape, M. J., 1993: Comparison of the chemiluminescence responses of bovine neutrophils to differently opsonized zymosan particles. *American Journal of Veterinary Research* **54**, 1055–1059.

Leino, L.; Sorvajarvi, K., 1992: CDllb is a calcium-dependent epitope in human neutrophils. *Biochemical and Biophysical Research Communications* **187**, 195–200.

Leitner, G.; Anug, A. M.; Merin, U.; Silanikove, N., 2007: Pregnancy obstructs involution stage II of the mammary gland in cows: general biological implications. *Nature Proceedings*. doi: hdl:10101/npre.2007.846.2.

Lemire, J. M.; Adams, J. S.; Kermani-Arab, V.; Bakke, A. C.; Sakai, R.; Jordan, S. C., 1985: 1, 25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity in vitro. *Journal of Immunology* **134**, 3032–3035.

Lotan, E.; Alder, J. H., 1976: Observations on the effect of shortening the dry period on milk yield, body weight, and circulating glucose and FFA levels in dairy cows. *Tijdschrift voor Diergeneeskunde* **101**, 77–82.

Madsen, T. G.; Andersen, J. B.; Ingvartsen, K. L.; Nielsen, M. O., 2004: Continuous lactation in dairy cows: effects on feed intake, milk production and mammary nutrient extraction. *Journal of Animal Feed Science* **13**, 503–506.

Makuza, S. M.; McDaniel, B. T., 1996: Effects of days dry, previous days open, and current days open on milk yields of cows in Zimbabwe and North Carolina. *Journal of Dairy Science* **79**, 702–709.

Marti, A.; Lazar, H.; Ritter, P.; Jaggi, R., 1999: Transcription factor activities and gene expression during mouse mammary gland involution. *Journal of Mammary Gland Biology and Neoplasia* **4**, 145–152.

Mayer, B.; Doleschall, M.; Bender, B.; Bartyik, J.; Bosze, Z.; Frenyó, L. V.; Kacskovics, I., 2005: Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. *Journal of Dairy Research* **72**, 107–112.

McDonald, J. S.; Anderson, A. J., 1981a: Total and differential somatic cell counts in secretions from noninfected bovine mammary glands: the early nonlactating period. *American Journal of Veterinary Research* **42**, 1360–1365.

McDonald, J. S.; Anderson, A. J., 1981b: Total and differential somatic cell counts in secretions from noninfected bovine mammary glands: the peripartum period. *American Journal of Veterinary Research* **42**, 1366–1368.

McGrath, M. F.; Collier, R. J.; Clemmons, D. R.; Busby, W. H.; Sweeny, C. A.; Krivi, G. G., 1991: The direct in vitro effect of insulin like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. *Endocrinology* **129**, 671–678.

Meglia, G. E.; Johannisson, A.; Petersson, L.; Persson Waller, K., 2001: Changes in some blood micronutrients, leukocytes and neutrophil expression of adhesion molecules in periparturient dairy cows. *Acta Veterinaria Scandinavica* **42**, 139–150.

Mendelson, J.; Multer, M. M.; Bernheim, J. L., 1977: Inhibition of human lymphocyte stimulation by steroid hormones: cytokinetic mechanisms. *Clinical and Experimental Immunology* **27**, 127–134.

Michal, J. J.; Heirman, L. R.; Wong, T. S.; Chew, B. P.; Frigg, M.; Volker, L., 1994: Modulatory effects of dietary, β-carotene on blood and mammary leukocyte function in periparturient dairy cows. *Journal of Dairy Science* **77**, 1408–1421.

Miller, R. H.; Paape, M. J.; Peters, R. R.; Young, M. D., 1990: Total and differential somatic cell counts and N-Acetyl-PD-glucosaminidase activity in mammary secretions during dry period. *Journal of Dairy Science* **73**, 1751–1755.

Miller, J. K.; Brzezinska-Slebodzinska, E.; Madsen, F. C., 1993: Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science* **76**, 2812–2823.

Monfardini, E.; Paape, M. J.; Wang, Y.; Capuco, A. V.; Husheem, M.; Wood, L.; Burvenich, C., 2002: Evaluation of L-selectin expression and assessment of protein tyrosine phosphorylation in bovine polymorphonuclear neutrophil leukocytes around parturition. *Veterinary Research* **33**, 1–5.

Moreira da Silva, F.; Burvenich, C.; Massart-Leen, A. M.; Russell-Pinto, F. F., 1997: Effect of some reproduction hormones on the oxidative burst of bovine neutrophils. *Flemish Veterinary Journal* **66**, 297–314.

Mori, T.; Kobayashi, H.; Hirohumi, H. H.; Suzuki, A.; Nishimura, T.; Mori, T., 1997: Inhibitory effect of progesterone and 20 ahyproxypregn-4-en-3-one on the phytohemagglutinin-induced transformation of human lymphocytes. *American Journal of Obstetrics and Gynecology* **127**, 151–157.

Murray, F. A.; Chenault, J. R., 1982: Effects of steroids on bovine T-lymphocyte blastogenesis in vitro. *Journal of Animal Science* **55**, 1132–1138.

Nagahata, H.; Nochi, H.; Tamoto, K.; Noda, H.; Kociba, G. J., 1995: Expression and role of adhesion molecule CD18 on bovine neutrophils. *Canadian Journal of Veterinary Research* **59**, 1–7.

Neave, F. K.; Dodd, F. H.; Henriques, E., 1950: Udder infection in the dry period. *Journal of Dairy Research* **17**, 37–49.

Newbould, F. H. S., 1973: The effect of added serum and glucose, and some inherent factors, on phagocytosis in vitro by milk leukocytes from several cows. *Canadian Journal of Comparative Medicine* **37**, 189–194.

Nguyen, D. D.; Neville, M. C., 1998: Tight junction regulation in the mammary gland. *Journal of Mammary Gland Biology and Neoplasia* **3**, 233–246.

Noble, M. S.; Hurley, W. L., 1999: Effect of secretion removal on bovine mammary gland function following an extended milk stasis. *Journal of Dairy Science* **82**, 1723–1730.

Nonnecke, B. J.; Smith, K. L., 1984: Biochemical and antibacterial properties of bovine mammary secretion during mammary involution and at parturition. *Journal of Dairy Science* **67**, 2863–2872.

Nonnecke, B. J.; Burton, J. L.; Kehrli, J. M. E., 1997: Associations between function and composition of blood mononuclear leukocyte populations from Holstein bulls treated with dexamethasone. *Journal of Dairy Science* **80**, 2403–2410.

Oliver, S. P.; Bushe, T., 1987: Growth inhibition of *Escherichia coli* and *Klebsiella pneumoniae* during involution of the bovine mammary gland: relation to secretion composition. *American Journal of Veterinary Research* **48**, 1669–1673.

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.; Smith, K. L., 1982a: Milk yield and secretion composition following intramammary infusion of colchicines. *Journal of Dairy Science* **65**, 204–210.

Oliver, S. P.; Smith, K. L., 1982b: Nonantibiotic approach in control of bovine mastitis during dry period. *Journal of Dairy Science* **65**, 2119–2124.

Oliver, S. P.; Sordillo, L. M., 1988: Udder health in the periparturient period. *Journal of Dairy Science* **71**, 2584–2606.

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., 1956: 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.

Ongsakul, M.; Sirisinha, S.; Lamb, A. J., 1985: Impaired blood clearance of bacteria and phagocytic activity of vitamin A deficient rats. *Proceedings of the Society for Experimental Biology and Medicine* **178**, 204–208.

Paape, M. J.; Guidry, A. J., 1977: Effect of fat and casein on intracellular killing of Staphylococcus aureus by milk leukocytes. *Proceedings of the Society for Experimental Biology and Medicine* **155**, 588–593.

Paape, M. J.; Guidry, A. J.; Jain, N. C.; Miller, R. H., 1991: Leukocytic defense mechanisms in the udder. *Flemish Veterinary Journal* **62**, 95–109.

Paape, M. J.; Capuco, A. V.; Guidry, A. J.; Burvenich, C., 1995: States morphology, function, and adaptation of mammary cells in normal and disease. *Journal of Animal Science* **73**, 1–17.

Paape, M. J.; Mehrzad, J.; Zhao, X.; Detilleux, J.; Burvenich, C., 2002: The defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. *Journal of Mammary Gland Biology and Neoplasia* **7**, 109–121.

Park, Y. H.; Fox, L. K.; Hamilton, M. J.; Davies, W. C., 1992: Bovine mononuclear leukocyte subpopulation in peripheral blood and mammary gland secretions during lactation. *Journal of Dairy Science* **75**, 998–1006.

Patton, S., 1974: Reversible suppression of lactation by colchicines. *FEBS Letters* **48**, 85–87.

Peaker, M.; Linzell, J. L., 1975: Citrate in milk: a harbinger of lactogenesis. *Nature* **253**, 464–465.

Pezeshki, A.; Mehrzad, J.; Ghorbani, G. R.; Rahmani, H. R.; Collier, R. J.; Burvenich, C., 2007: Effects of short dry periods on performance and metabolic parameters in Holstein dairy cows. *Journal of Dairy Science* **90**, 5531–5541.

Pezeshki, A.; Mehrzad, J.; Ghorbani, G. R.; De Spiegeleer, B.; Collier, R. J.; Burvenich, C., 2008: The effect of dry period length reduction to 28 days on the performance of multiparous dairy cows in the subsequent lactation. *Canadian Journal of Animal Science* **88**, 449–456.

Pezeshki, A.; Vergote, V.; Van Dorpe, S.; Baert, B.; Burvenich, C.; Popkov, A.; De Spiegeleer, B., 2009: Adsorption of peptides at the sample drying step: influence of solvent evaporation technique, vial material and solution additive. *Journal of Pharmaceutical and Biomedical Analysis* **49**, 607–612.

Preisler, M. T.; Weber, P. S.; Tempelman, R. J.; Erskine, R. J.; Hunt, H.; Burton, J. L., 2000: Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. *American Journal of Veterinary Research* **61**, 14–19.

Putnam, M. E.; Comben, N., 1987: Vitamin E. *The Veterinary Record* **121**, 541–545.

Quarrie, L. H.; Addey, C. V. P.; Wilde, C. J., 1996: Programmed cell death during mammary involution induced by weaning, litter removal, and milk stasis. *Journal of Cellular Physiology* **168**, 559–569.

Rainard, P.; Riollet, C., 2006: Innate immunity of the bovine mammary gland. *Veterinary Research* **37**, 369–400.

Rajala-Schultz, P. J.; Hogan, J. S.; Smith, K. L., 2005: Short communication: association between milk yield at dry-off and probability of intramammary infections at calving. *Journal of Dairy Science* **88**, 577–579.

Rastani, R. R.; Grummer, R. R.; Bertics, S. J.; Gumen, A.; Wiltbank, M. C.; Mashek, D. G.; Schwab, M. C., 2005: Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *Journal of Dairy Science* **88**, 1004–1014.

Reichel, H.; Koeffler, H. P.; Tobler, A.; Norman, A. W., 1987: 1-alpha,25-Dihydroxyvitamin D 3 inhibits gamma- interferon synthesis by normal human peripheral blood lymphocytes. *Proceedings of the National Academy of Sciences of the USA* **84**, 3385–3389.

Reid, I. M.; Roberts, C. J.; Treacher, R. J.; Williams, L. A., 1986: Effect of body condition at calving on tissue mobilization, development of fatty liver and blood chemistry of dairy cows. *Animal Production* **43**, 7–15.

Reiter, B.; Brock, J. H.; Steel, E. D., 1975: Inhibition of *Escherichia coli* by bovine colostrum and post-colostral milk II. The bacteriostatic effect of lactoferrin on a serum susceptible and serum resistant strain of *E. coli*. *Immunity* **28**, 83–95.

Rejman, J. J.; Hegarty, H. M.; Hurley, W. L., 1989: Purification and characterization of bovine lactoferrin from secretions of the involuting mammary gland: identification of multiple molecular weight forms. *Comparative Biochemistry and Physiology-Part B* **93**, 929–934.

Remond, B.; Bonnefoy, J. C., 1997: Performance of a herd of Holstein cows managed without the dry period. *Annales de Zootechnie* **46**, 3–12.

Remond, B.; Kerouanton, J.; Brocard, V., 1997a: Effets de la reduction de la duree de la periode seche ou de son omission sur les performances des vaches laitie'ras. *INRA Productions Animales* **10**, 301–333.

Remond, B.; Rouel, J.; Pinson, N.; Jabet, S., 1997b: An attempt to omit the dry period over three consecutive lactations in dairy cows. *Annales de Zootechnie* **46**, 399–408.

Rémond, B.; Ollier, A.; Miranda, G., 1992: Milking cows in late pregnancy: milk production during this period and during the succeeding lactation. *Journal of Dairy Research* **59**, 233–241.

Rinaldi, M.; Li, R. W.; Bannerman, D. D.; Daniels, K. M.; Evock-Clover, C.; Silva, M. V. B.; Paape, M. J.; Van Ryssen, B.; Burvenich, C.; Capuco, A. V., 2009: A sentinel for teat tissues in dairy cows: dominant innate immune response elements define early response to *E. Coli* mastitis. *Functional and Integrative Genomics*. doi: 10.1007/s10142-009-0133-z.

Roth, J. A.; Flaming, K. P., 1990: Model systems to study immunomodulation in domestic food animals. *Advances in Veterinary Science and Comparative Medicine* **35**, 21–41.

Roth, J. A.; Kaeberle, M. L.; Hsu, W. H., 1982a: Effect of estradiol and progesterone on lymphocyte and neutrophil functions in steers. *Infection and Immunity* **35**, 997–1002.

Roth, J. A.; Kaeberle, M. L.; Hsu, W. H., 1982b: Effects of ACTH administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis. *American Journal of Veterinary Medicine* **43**, 412–416.

Roth, J. A.; Kaeberle, M. L.; Appell, L. H.; Nachreiner, R. F., 1983: Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. *American Journal of Veterinary Research* **44**, 247–253.

Rukkwamsuk, T.; Kruip, T. A.; Meijer, G. A. L.; Wensing, T., 1999: Hepatic fatty acid composition in periparturient dairy cows with fatty liver induced by intake of a high energy diet in the dry period. *Journal of Dairy Science* **82**, 280–287.

Russell, M. W.; Reiter, B., 1975: Phagocytic deficiency of bovine milk leukocytes: an effect of casein. *Journal of the Reticuloendothelial Society* **18**, 1–13.

Russell, M. W.; Brooker, B. E.; Reiter, B., 1976: Inhibition of the bactericidal activity of bovine polymorphonuclear leukocytes and related systems by casein. *Research in Veterinary Science* **20**, 30–35.

Saad, A. M.; Concha, C.; Astrom, G., 1989: Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. *Journal of Veterinary Medicine* **36**, 337–345.

Sanders, H. G., 1928: The variations in milk yields caused by season of the year, service, age, and dry period, and their elimination. Part IV. Dry period and standardization of yields. *The Journal of Agricultural Science* **18**, 209–251.

Sartorelli, P.; Paltrinieri, S.; Agnes, F., 1999: Non-specific immunity and ketone bodies. I: in vitro studies on chemotaxis and phagocytosis in ovine neutrophils. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine* **46**, 613–619.

Sartorelli, P.; Paltrinieri, S.; Comazzi, S., 2000: Non-specific immunity and ketone bodies. II: In vitro studies on adherence and superoxide anion production in ovine neutrophils. *Journal of Veterinary Medicine A* **47**, 1–8.

Schairer, M. L., 2001: Estrogen treatments for the initiation of dry off in dairy cows. Master thesis, University of Florida, Gainesville, FL, USA.

Schanbacher, F. L.; Goodman, R. E.; Talhouk, R. S., 1993: Bovine mammary lactoferrin: implications from messenger ribonucleic acid (mRNA) sequence and regulation contrary to other milk proteins. *Journal of Dairy Science* **76**, 3812–3831.

Shafer-Weaver, K. A.; Pighetti, G. M.; Sordillo, L. M., 1996: Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. *Proceedings of the Society for Experimental Biology and Medicine* **212**, 271–279.

Siiteri, P. K.; Febres, F.; Clemens, L. E.; Chang, R. J.; Gondos, B.; Stites, D. P., 1977: Progesterone and the maintenance of pregnancy: is progesterone nature's immunosuppressant? *Annals of the New York Academy of Sciences* **286**, 384–397.

Smith, A.; Wheelock, J. V.; Dodd, F. H., 1967: Effect of milking throughout pregnancy on milk secretion in the succeeding lactation. *Journal of Dairy Research* **34**, 145–150.

Smith, K. L.; Harrison, J. H.; Hancock, D. D.; Todhunter, D. A.; Conrad, H. R., 1984: Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. *Journal of Dairy Science* **67**, 1293–1300.

Smith, K. L.; Todhunter, D. A.; Schoenberger, P. S., 1985a: Environmental pathogens and intramammary infection during the dry period. *Journal of Dairy Science* **68**, 402–417.

Smith, K. L.; Todhunter, D. A.; Schoenberger, P. S., 1985b: Environmental mastitis: cause, prevalence, prevention. *Journal of Dairy Science* **68**, 1531–1553.

Smith, K. L.; Todhunter, D. A.; Schoenberger, P. S., 1985c: Lactoferrin and defense of the involuted mammary gland against infection by environmental pathogens. *Kieler Milchwirtschaft Forschungsber* **37**, 477–481.

Smith, K. L.; Hogan, J. S.; Weiss, W. P., 1997: Dietary vitamin E and selenium affect mastitis and milk quality. *Journal of Animal Science* **75**, 1659–1665.

Sordillo, L. M., 1987: Physiological aspects of bovine mammary involution: a biochemical and morphological investigation. PhD Dissertation, Louisiana State University, Baton Rouge, LA, USA.

Sordillo, L. M., 2005: Factors affecting mammary gland immunity and mastitis susceptibility. *Livestock Production Science* **98**, 89–99.

Sordillo, L. M.; Nickerson, S. C., 1988: Morphologic changes in the bovine mammary gland during 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.

Stein, T.; Morris, J. S.; Davies, C. R.; Weber-Hall, S. J.; Duffy, M. A.; Heath, V. J.; Bell, A. K.; Ferrier, R. K.; Sandilands, G. P.; Gusterson, B. A., 2004: Involution of the mouse mammary gland is associated with an immune cascade and an acute-phase response, involving LBP, CD14 and STAT3. *Breast Cancer Research* **6**, R75–R91.

Suriyasathaporn, W.; Heuer, C.; Noordhuizen-Stassen, E. N.; Schukken, Y. H., 2000: Hyperketonemia and the impairment of udder defense: a review. *Veterinary Research* **31**, 397–412.

Swanson, E. W., 1965: Comparing continuous milking with sixty-day dry periods in successive lactations. *Journal of Dairy Science* **48**, 1205–1209.

Swanson, E. W.; Pardue, F. E.; Longmire, D. B., 1967: Effect of gestation and dry period on deoxyribonucleic acid and alveolar characteristics of bovine mammary glands. *Journal of Dairy Science* **50**, 1288–1292.

Targowski, S. P.; Klucinski, W., 1983: Reduction in mitogenic response of bovine lymphocytes by ketone bodies. *American Journal of Veterinary Research* **44**, 828–830.

Tatarczuch, L.; Philip, C.; Lee, C. S., 1977: Involution of sheep mammary gland. *Journal of Anatomy* **190**, 405–416.

Tjoelker, L. W.; Chew, B. P.; Tanaka, T. S.; Daniel, L. R., 1988: Bovine vitamin A and β-carotene intake and lactational status. 1. Responsiveness of peripheral blood polymorphonuclear leukocytes to vitamin A and β-carotene challenge in vitro. *Journal of Dairy Science* **71**, 3112–3119.

Tjoelker, L. W.; Chew, B. P.; Tanaka, T. S.; Daniel, L. R., 1990: Effect of dietary vitamin A and β-Carotene on

polymorphonuclear leukocyte and lymphocyte function in dairy cows during the early dry period. *Journal of Dairy Science* **73**, 1017–1022.

Travers, M. T.; Barber, M. C.; Tonner, E.; Quarrie, L.; Wilde, C. J.; Flint, D. J., 1996: The role of prolactin and growth hormone in the regulation of casein gene expression and mammary cell survival: relationships to milk synthesis and secretion. *Endocrinology* **137**, 1530–1539.

Van Merris, V.; Meyer, E.; Duchateau, L.; Burvenich, C., 2004: Differential effects of steroids and retinoids on bovine myelopoiesis in vitro. *Journal of Dairy Science* **87**, 1188–1195.

Vangroenweghe, F.; Lamote, I.; Burvenich, C., 2005: Physiology of the periparturient period and its relation to severity of clinical mastitis. *Domestic Animals Endocrinology* **29**, 283–293.

Watson, D. L.; Brandon, M. R.; Lascelles, A. K., 1972: Concentration of immunoglobulin in mammary secretion of ruminants during involution with particular reference to selective transfer of IgG_1. *Australian Journal of Experimental Biology and Medical Science* **50**, 535–539.

Watters, R. D.; Guenther, J. N.; Brickner, A. E.; Rastani, R. R.; Crump, P. M.; Clark, P. W.; Grummer, R. R., 2008: Effects of dry period length on milk production and health of dairy cattle. *Journal of Dairy Science* **91**, 2595–2603.

Weisdorf, D. J.; Craddock, P. R.; Jacob, H. S., 1982: Granulocytes utilize different energy sources for movement and phagocytosis. *Inflammation* **6**, 245–256.

Weiss, W. P., 2002: Relationship of mineral and vitamin supplementation with mastitis and milk quality. In: *Proceedings of Annual Meeting, National Mastitis Council,* Orlando, FL, USA. Madison, WI: National mastitis council, pp. 37–44. Available at: http://www.nmconline.org/articles/nutr.pdf

Weiss, W. P.; Hogan, J. S.; Smith, K. L.; Hoblet, K. H., 1990: Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. *Journal of Dairy Science* **73**, 381–390.

Wentink, G. H.; Rutten, V. P. M.; Van den Ingh, T. S.; Hoek, A.; Muller, K. E.; Wensing, T., 1997: Impaired specific immunoreactivity in cows with hepatic lipidosis. *Veterinary Immunology and Immunopathology* **56**, 77–83.

Wilde, C. J.; Addey, C. V. P.; Li, P.; Fernig, D. G., 1997: Programmed cell death in bovine mammary tissue during lactation and involution. *Experimental Physiology* **82**, 943–953.

Wilde, C. J.; Knight, C. H.; Flint, D. J., 1999: Control of milk secretion and apoptosis during mammary involution. *Journal of Mammary Gland Biology and Neoplasia* **4**, 129–136.

Winters, K. R. H.; Meyer, E.; Van Merris, V. M.; Van Den Broeck, W. L. M.; Duchateau, L.; Burvenich, C., 2003: Sex steroid hormones do not influence the oxidative burst activity of polymorphonuclear leukocytes from ovariectomized cows in vitro. *Steroids* **68**, 397–406.

Wyle, F. A.; Kent, J. R., 1977: Immunosuppression by sex steroid hormones I. The effect upon PHA- and PPD-stimulated lymphocytes. *Clinical and Experimental Immunology* **27**, 407–415.

Xin, Z.; Waterman, D. F.; Hemken, R. W.; Harmon, R. J., 1991: Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. *Journal of Dairy Science* **74**, 3078–3085.