New Developments in Mastitis Research

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Take Home Messages

- The need to decrease the use of antibiotics forces the pharmaceutical industry to create new, innovative solutions
- Improvement of immune status of the fresh cow may reduce susceptibility to mastitis
- Lower milk production at dry off decreases the risk of new intramammary infections during the dry period
- Information about the microbiome of the udder and the bacterial load give new insights into the consequences of antimicrobial treatment
- Selective dry cow treatment has been proven to be feasible without adverse effects on udder health

New Developments from the Pharmaceutical Industry

Over the last ten years, there has been a strong focus on the prudent use of antimicrobials. WHO decided that in the future all new developed antibiotics will only be permitted for human medicine and not for veterinary medicine. This means, that we have to look for new solutions in order to keep our cows healthy or to treat them in different ways, with less or no antibiotics.

Recently, two innovative treatments have been developed:

a. Immuno-modulation: The dairy industry has its first non-antibiotic product that boosts the immune system. It’s a protein that boosts the dairy cow’s natural immune system during the critical time around calving.

b. Abrupt dry-off: The second innovative treatment is a product that induces abrupt dry-off in dairy cows. In combination with selective dry cow treatment, it should diminish milk leaking, risk of new intramammary infections during the dry period, and increase animal welfare.

Immuno-modulation

Dairy cows often experience decreased immune function around the time of calving, typified by impaired polymorphonuclear neutrophil (PMN; white blood cell) function and a transient neutropenia. This is associated with increased disease incidence, including mastitis, retained placenta, and metritis. This decreased cell function often starts about 2-3 weeks before calving, reaching a nadir at the time of calving (0-2 days after calving), and then recovering in 2-4 weeks (Kehrli et al., 1989). The decreased immune function during the periparturient period is more pronounced in some cows than in others. The decreased immune function has been attributed to fetal growth and colostrum production during the last part of the pregnancy, which induces an increased nutrient demand (Goff et al., 2002). At the start of lactation most cows experience a negative energy and protein balance, as well as changes in vitamin and mineral status which contributeto a decreased PMN function. Supplementation of vitamins and minerals may increase PMN function and decrease disease incidence.

Recently, it was shown that a subcutaneous injection of recombinant bovine granulocyte stimulating factor (rbG-CSF) increased the number of PMN and improved cytotoxicity function by PMN (Kimura et al., 2014). Granulocyte colony-stimulating factor is a glycoprotein cytokine that stimulates the bone marrow to produce stem cells and granulocytes, and release granulocytes into the bloodstream. This increased number of mature and immature granulocytes should improve the immune system of the fresh cow and decreases periparturient diseases.

Abrupt Dry-off

The dry period is an important phase of a dairy cow's lactation cycle and studies clearly document the importance of a dry period lasting 40 to 60 d for optimal milk production. The overall benefits derived from the dry period are thought to be a consequence of adequate mammary gland secretory cell turnover. Abrupt cessation of lactation has become problematic for the modern dairy cow, which may produce 25 to 30 kg/d of milk at the time of dry-off.

Excessive accumulation of milk at the time of dry-off, may lead to milk leakages and reduce the functional capabilities of mammary leukocyte populations (Oliver and Sordillo, 1989) and make cows therefore more susceptible to new intramammary infection during the early dry period.

Fully involuted mammary glands are more resistant to new bacterial infections and mammary glands that enter the dry period with lower milk production involute at a faster rate than those of cows with a high milk production. On top of that, excessive mammary gland engorgement at the time of dry-off can be seen as a source of discomfort and pain.

In a time where the use of blanket antimicrobial dry cow treatment (BDCT) is no longer a standard in Europe, it is even more important to improve transition management in order to create the most favourable circumstances for dry off cows. In the guideline “The use of antimicrobials for drying off dairy cows,” published in January 2012 in the Netherlands, recommendations are given to decrease milk production before drying off to 12 kg of milk per cow per day. The dairy industry needs practical management strategies to effectively dry off high-producing dairy cows at the end of their lactations. This can be achieved through feed restrictions and decreased protein content at the end of the lactation.

Feed restrictions, however, have several disadvantages. It has been reported that cows exposed to reduced feed intake had a greater frequency of vocalization and probably suffered from hunger. Furthermore, severe feed restriction caused increased cortisol levels, affected non-esterified fatty acid, BHB, and urea concentrations. All these metabolic imbalances indicate a negative energy balance that might cause an impairment of udder defense mechanisms.

Recently, some pharmacological studies explored options to hasten mammary gland involution, like the systemic application of prolactin-release inhibitors. The effect of those inhibitors on mammary gland involution has been investigated. Prolactin-release inhibitors reduce the release of prolactin in the pituitary gland. As a result, the galactopoietic (=milk forming) effect of prolactin is countered.

Cabergoline is an ergot derivative and acts as a prolactine-relase inhibitor. A recent study reported that a single injection of cabergoline is able to reduce plasma prolactine concentrations in dairy cows at dry-off. In addition, the cabergoline treatment at dry-off also reduced udder engorgement (measured by a digital algometer Identifying pressure), decreased the incidence of milk leakage, and improved lying time the day following dry-off (Bach et al., 2015).

Large field trials were conducted in Europe, with positive results shown regarding decreased milk leakage and fewer new intramammary infections. After the official launch of the product on the market, unfortunately, side effects were seen in a few European countries, with symptoms that looked similar to milk fever (lying down and being unable to stand). Many of these affected cows ended up as Downer cows and some of them died. Because of these negative side effects the European Medicine Agency (EMA) recommended that the marketing authorisation for Velactis© be suspended in the European Union (EU) until further information is available to show that the benefits outweigh the risks, possibly under new conditions of use or restrictions. At this moment, new trials are ongoing in Europe and new results are expected soon. Other countries, like Mexico and Brazil, don't report about those side effects and the product is still allowed to be used in those countries.

▪ The Microbiome of the Udder

For years now, mammary gland inflammation has been considered the result of host pathogen interaction, a result that depends notably on bacterial and host genetic determinants. The immunological response of the mammary gland on invading bacteria is an increase in somatic cell count (SCC). Somatic cell count (SCC) has therefore been used to distinguish healthy quarters from quarters with an inflammatory response, most likely due to an intramammary infection. Also, bulk milk somatic cell counts (BMSCC) are used as an overall indicator of milk quality.

Identification of the bacteria responsible for mastitis is an important component of the eventual clinical resolution of the disease. Currently, bacterial culture is the gold standard method for identification of mastitis-causing microorganisms. Classical bacterial culture needs about 24-48 hours to obtain results, and in approximately 25% of milk samples from clinical mastitis cases, bacteria are not detected or present.

In the last decade new molecular techniques have been used to diagnose mastitis. These techniques are being used to identify bacterial DNA in milk samples. In a recent study, the use of DNA techniques showed the diversity of bacterial communities in human milk samples. Furthermore, they showed that this technique identified a much greater diversity of bacteria in milk than that previously reported in culture-dependent studies.

These DNA-based bacteriological studies were also conducted in milk from dairy cows (Oikonomou et al. 2012). The concept that the mammary gland and milk were sterile, however, has to be interpreted in a different perspective. The DNA-based studies showed a wide variety of micro-organisms in the milk, even in samples from healthy, low SCC quarters. These data led to the concept that intramammary microbiota are composed of a complex community of diverse bacteria. Accordingly, mammary gland infections are not infections by bacteria, but rather a consequence of dysbacteriosis or dysbiosis.

One hundred and thirty-six milk samples were collected from cows with subclinical or clinical mastitis and sent to the lab for bacteriological culture (Oikonomou et al., 2012). Another 20 milk samples obtained from healthy quarters without a history of mastitis and SCC lower than 10,000 cells/ml were also used. No Mycoplasma culture or anaerobic culture was performed on the samples. Samples with a single dominant bacteria or absence of any growth were selected for the study. Results showed that the microbiota from the healthy quarters were quite different from the mastitis milk samples. The presence of bacteria that don't need oxygen to grow, the so called anaerobic bacteria, were found in high proportions by the DNA-based technique. In this study, it was not possible to differentiate between cause and consequence, but it was concluded that a wide variety of udder commensals exist that play a crucial role in the mastitis aetiology. These new findings might have future consequences for the current thoughts about the prevention and cure of mastitis. If we can divide bacteria in the udder into "good guys and bad guys," will this affect our current prevention strategies? Will post milking teat dipping or blanket dry cow treatment still be the best way to go, or might we also kill "the good guys"? Future research will help us to answer these questions.

▪ Implementation of Selective Dry Cow Treatment

An overview of the Dutch national BMSCC over the last 11 years is shown in Figure 1. BMSCC shows a decreasing trend and BMSCC dropped below 200,000 cells/ml in 2015. Since the ban on the preventive use of antimicrobials in 2012 and the introduction of selective dry cow treatment (SDCT) in January 2014, national BMSCC declined by 30,000 cells/ml. As of June 2017, the national BMSCC is the lowest ever.

Somatic cell count dynamics during the dry period were calculated for the 280 dairy herds belonging to the University Farm Animal Practice in the Netherlands. The rate of new intramammary infections during the dry period and the cure rate of existing intramammary infections during the dry period were calculated by the national DHI (CRV), based on the following definitions:

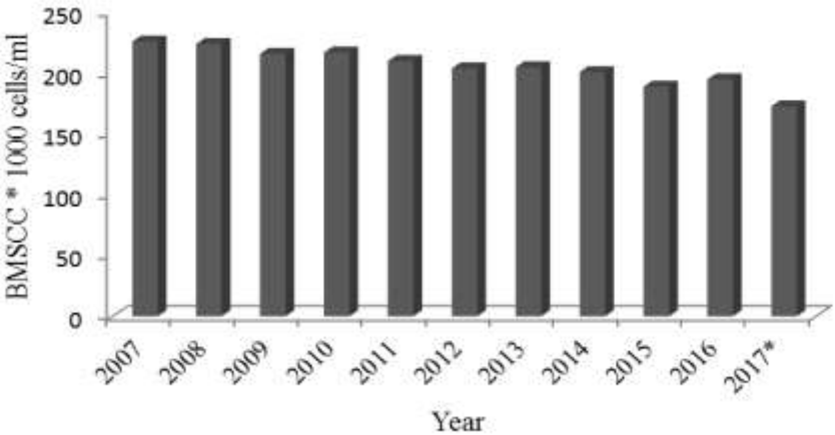

Figure 1. National BMSCC in the Netherlands from 2007 until 2017 (n= approximately 18,000 herds). *Average BMSCC of 2017 is calculated until June 2017.

New intramammary infection (IMI):

Defined as a change in SCC from below the threshold of 150,000 cells/ml for primiparous and 250,000 cells/ml for multiparous cows at the last milk recording before calving to an SCC equal to or greater than the threshold at the first milk recording after calving.

Cured intramammary infection (IMI):

Defined as a change in SCC from equal to or greater than the threshold (150,000 cells/ ml for primiparous and 250,000 cells/ml for multiparous cows) at the last milk recording before calving to an SCC below the threshold at the first milk recording after calving.

Results of these SCC changes during the dry period are shown in Table 1. The annual mean % of new IMI changed from 16% in 2013 to 18% in 2014 and to 17% in 2016, a slight increase in the number of new infections during the dry period. The annual mean % for cured IMI during the dry period was 74% in 2013 and 2014, and 75% in 2017.

Table 1. Somatic cell count dynamics over the dry period from 2013 through 2017 in herds serviced by the University Farm Animal Practice in the Netherlands (n= 280 herds). Thresholds for primiparous cows 150,000 cells/ml and 250,000 cells/ml for multiparous cows

	Year				
Variable	2013	2014	2015	2016	2017*
Mean (SD) % new IMI	16 (9)	18 (10)	17 (9)	17 (10)	15 (8)
Mean (SD) % cured IMI	74 (16)	74 (18)	76 (7)	75 (18)	75 (19)

* calculations for 2017 are carried out until December 1st 2017

Following a period in which BDCT was an essential part of the of the 5-point mastitis control plan, the results from this study indicate that in the Netherlands a nationwide forced shift to SDCT over a relatively short period of time was associated with no significant changes to udder health during the dry period. National figures on BMSCC are lower than ever and there is no significant increase in the percentage of new intramammary infections during the dry period. Despite the investment of extra labour, farmers have become convinced that improving management works better than buying the solution in a tube or a syringe!

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