J. Dairy Sci. 100:1–13
https://doi.org/10.3168/jds.2016-12087

Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution

S. Lanctôt,* P. Fustier,† A. R. Taherian,† B. Bisakowski,† X. Zhao,* and P. Lacasse‡[1]
*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada H9X 3V9
†Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, Quebec, Canada J2S 8E3
‡Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada J1M 0C8

ABSTRACT

The transition from lactation to the dry period in dairy cows is a period of high risk for acquiring new intramammary infections. This risk is reduced when the involution of the mammary gland is completed. Accordingly, approaches that speed up the involution process after drying-off could reduce the incidence of mastitis. The current study aimed to develop a biological response modifier that could be injected into cow teats to promote immune cell migration and speed up involution. Chitosan, a natural polysaccharide derived from chitin, is able to trigger host innate immunity. We developed 2 formulations made from either high- or low-viscosity chitosan. Both are liquid at room temperature but form a hydrogel at body temperature. In the first experiment, each udder quarter of 7 Holstein cows in late lactation was randomly assigned at drying-off to receive one of the following intramammary infusions: 2.5 or 5 mL of 5% (wt/vol) low-viscosity chitosan hydrogel, 5 mL of 5% high-viscosity chitosan hydrogel, or 5 mL of water. Milk (mammary secretion) samples were collected from each quarter on d −4, −1 (drying-off), 1, 3, 5, 7, and 10. Milk somatic cell counts and the concentrations of involution markers such as BSA, lactate dehydrogenase, and lactoferrin were measured in each sample. In comparison with the control, the chitosan hydrogel infusions significantly hastened the increases in somatic cell counts, BSA and lactoferrin concentrations, and lactate dehydrogenase activity in mammary secretions. No major differences between sources or volumes of chitosan were observed for the measured parameters. The compatibility of this approach with an internal teat sealant was verified in the second experiment. Each udder quarter of 8 Holstein cows was randomly assigned at drying-off to receive one of the following intramammary infusions: 5 mL of 2% low-viscosity chitosan hydrogel, 4 g of an internal teat sealant, a combination of sealant and chitosan, or 5 mL of water. Milk (mammary secretion) samples were collected from each quarter on d −4, −1 (drying-off), 5, and 10 to measure involution markers. These results suggest that chitosan hydrogel infusion hastened mammary gland involution and activate immune response, which may reduce the risk of acquiring new intramammary infections during the drying-off period. Those results were not affected by the presence of the teat sealant, showing that both approaches are fully compatible and could be used in combination.

Key words: mastitis, involution, immunity, chitosan, dairy cow

INTRODUCTION

The lactation cycle of a dairy cow must include a dry period for optimal milk production in the following lactation (Andersen et al., 2005). Although milking cessation is essential for proper cell renewal, during early involution the cow mammary gland is vulnerable to new IMI (Smith et al., 1985; Leelahapongsathon et al., 2016). Even though milking is stopped, high-yielding cows still secrete a significant amount of milk. The pressure buildup causes milk to leak and impairs keratin formation (Dingwell et al., 2004). Once the teat canal is open, microorganisms gain access to the mammary gland and cause infection (Cousins et al., 1980). Moreover, during early involution, the level of antibacterial components and concentration of immune cells in the milk secretions are minimal (Sordillo et al., 1987). Finally, high fat, casein, and lactose concentrations favor bacterial growth and interfere with the phagocytosis capacity of immune cells (Sordillo and Nickerson, 1988). Therefore, implementing an efficient mastitis prevention program during this period is crucial.

An important element of many mastitis control programs is the treatment of all cows with antibiotics at the end of lactation, regardless of the cows' infection status (Berry and Hillerton, 2002). Although this method aims to cure existing infections and prevent new IMI during the dry period, it is not equally ef-

Received September 30, 2016.
Accepted November 26, 2016.
[1]Corresponding author: Pierre.Lacasse@agr.gc.ca

fective against all pathogens (Oliver et al., 2011). The major concern, perhaps, is consumer perception. As a result, Germany and the Netherlands have prohibited the prophylactic use of antibiotics in livestock, such that only cows with IMI can be treated (Swinkels et al., 2015). Consequently, the need is increasing for effective nonantibiotic IMI prevention treatments. Internal teat sealants could provide alternatives to dry-cow therapy. However, despite their benefits, the inert bismuth-based preparations are not totally effective (Krömker et al., 2014). Another proposed alternative to blanket dry-cow therapy is external teat-dipping regimens using iodine-based teat dips at drying-off. Despite their effectiveness in tie-stall herds, these regimens are labor intensive and difficult to implement in free-stall operations (Whist et al., 2006).

When early involution of the mammary gland is completed, the risk of acquiring a new IMI is minimal (Tatarczuch et al., 2002). Consequently, Oliver and Smith (1982) proposed that accelerating the involution process after drying-off could enhance the resistance of the mammary gland to new IMI during early involution. This acceleration can be achieved by using a biological response modifier (**BRM**; Tzianabos, 2000). However, the effect of the BRM tested so far are of short duration (Shamay et al., 2003; Dallard et al., 2010). Chitosan is a natural biocompatible polysaccharide derived by the partial deacetylation of chitin, which is the second most abundant polysaccharide in nature after cellulose (Rinaudo, 2006). Chitosan can be formulated to be injectable at room temperature but form a biodegradable hydrogel at body temperature (Chenite et al., 2000). Chitosan exhibits various biological properties. It has been used for drug formulation over the past 20 yr. Moreover, chitosan has bacteriostatic, bioadhesive, and bioactive properties (Şenel and McClure, 2004).

After drying-off, the permeability of tight junctions between epithelial cells increases, which allows paracellular transport between the interstitial space and milk (Nguyen and Neville, 1998). This transport can be assessed by measuring concentration of serum albumin and immunoglobulin in milk secretions (Hurley, 1989). Furthermore, the regression of mammary secretory tissue is accompanied by changes in milk secretion composition that occur gradually during early involution (Oliver and Sordillo, 1989). For instance, epithelial cells produce more lactoferrin as involution progresses (Capuco and Akers, 1999). Accordingly, milk secretion concentration of those markers is used to assess mammary gland involution progress.

The present study aimed to develop a chitosan-based BRM formulation that could be injected into the cow teat to promote sustained immune cell migration and hasten involution at drying-off. In addition, the effect of combining the chitosan-based BRM with an internal teat sealant was evaluated.

MATERIALS AND METHODS

Preparation of Treatments

All treatments were prepared with aseptic, nonpyrogenic products and materials under a laminar flow hood. For each concentration of chitosan (2% and 5% wt/vol), a 200-mL solution was made by adding 120 mL of nonpyrogenic water (<0.005 endotoxin units/mL; Lonza, Walkersville, MD) to preweighed chitosan. The solution was agitated at 200 rpm with a metal mixing rod. The pH of the solution was reduced to 3 via the addition of 0.1 *M* HCl (Sigma-Aldrich Co., St. Louis, MO). The preparation was kept overnight at room temperature for complete hydration. The following day, the preparation pH was brought up to 6.8 using a 50% (wt/vol) β-glycerophosphate disodium salt hydrate (Sigma-Aldrich Co.) solution. Then, the volume was adjusted to 200 mL by the addition of nonpyrogenic water (Lonza). Finally, plastic syringes were filled with the desired volume, sealed with a cap, and stored at room temperature. We developed 2 formulations, using either high-viscosity (130-centipoise) or low-viscosity (90-centipoise) chitosan provided by Qingdao Yuda Century Economy and Trade Co. (Shibei District, Qingdao, China).

Animals and Experimental Design

The experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993). The cows were housed in individual tie stalls at Agriculture and Agri-Food Canada's Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada).

Experiment 1. Seven Holstein cows in late lactation (319 ± 29 DIM at drying off; ±SEM) producing more than 15 kg (average 22.6 ± 1.9) of milk per day were used. Cows were milked twice a day and projected or real 305-d milk production was 9312 ± 749 kg. The group of cows was dried off at the same time, 90 ± 17 d before the expected calving date. Prior to dry-off (d –4), quarter SCC averaged 122,693 ± 34,520 cell/mL. Until dry-off, the cows were fed ad libitum a late-lactation diet. After drying off, the cows were fed ad libitum a dry period diet and dry hay. Water was available ad libitum during the whole experiment.

At drying-off, each udder quarter was randomly assigned to 1 of 4 intramammary infusions, as follows: 5 mL of nonpyrogenic water (Lonza; n = 7), 2.5 mL of 5% (wt/vol) low-viscosity chitosan solution (**LV2.5**; n