

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

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### Why is this important?

For optimal milk production in the following lactation, dairy cows should be given a 60-day dry period. Milking cessation is essential for proper cell renewal, but early during involution of the udder is a period in which the udder is especially vulnerable to intramammary infections (IMI). This is because, even though milking has stopped, high-yielding cows still produce a significant amount of milk in those early days. The pressure buildup causes milk to leak and impairs formation of the keratin plug. With the teat canal remaining open, microbes can easily gain access to the udder and cause infection. To further exacerbate the problem, during early involution, the level of antibacterial components and concentration of immune cells in the milk are minimal. Finally, high fat, casein, and lactose concentrations favour bacterial growth. It is truly the perfect storm!

Producers have many different mastitis prevention programs to consider, including the use of blanket treatment of all cows with antibiotics at the end of lactation. However, with the increasing concern around antimicrobial resistance, it is important to explore non-antibiotic IMI prevention treatments. For instance, internal teat sealants can provide alternatives to dry-cow therapy, or animals can be selected for antibiotic treatment based on their past or current IMI status (selective dry-cow therapy). With early involution of the udder, the risk of acquiring a new IMI is minimal. Therefore, accelerating this process after drying-off could enhance the resistance of the udder to new IMI.

Chitosan is a natural biocompatible compound that can be formulated to be injectable at room temperature but forms a biodegradable hydrogel at body temperature. This compound exhibits various biological properties that may speed up involution of the udder and potentially reduce IMI. This study aimed to develop a chitosan-based formulation that could be injected into the cow's teat to promote immune cell movement into the udder and speed up udder involution at drying-off.

*Chitosan: a sugar that is obtained from the hard outer skeleton of shellfish, including crab, lobster, and shrimp.*

### What did we do?

The authors developed two formulations, using either high-viscosity or low-viscosity chitosan, and conducted two experiments, with the first experiment examining only chitosan treatment and the second experiment examining chitosan used in conjunction with Orbeseal teat sealant. Cows selected for the study were producing more than 15 kg/d and were dried off at the same time. Milk samples were collected and used to measure SCC, bacteria, and biological markers indicating involution and immune response.

### What did we find?

Researchers measured the inflammation of the udder following treatment and found that all quarters treated with chitosan were slightly more inflamed, although still mild and not lasting more than 24 hours.

In both experiments, for up to 5 days after dry-off, the concentration of measured immune and involution markers was higher in quarters treated with chitosan when compared to controls, indicating that mammary immune defense was likely improved and that mammary involution was occurring more quickly. In both experiments, SCC in milk increased faster in all chitosan-treated quarters than in the control quarters. The number of somatic cells increases in milk during the early involution period, and these cells act to intercept bacteria before they reach the mammary gland. The intramammary infusion of pro-inflammatory agents (such as chitosan) increases the number of somatic cells in the gland, thereby improving the chance that bacteria will be intercepted.

To provide optimal protection against pathogens, the mammary gland immune system needs to be activated. Once activation has occurred, the expression of immunoregulatory genes by udder immune and skin cells increases and these cells produce and release compounds that increase their bactericidal capacity. The chitosan hydrogel increased the expression of immunoregulatory genes by somatic cells, thereby providing further evidence that immune defense was improved by this treatment.

*Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enable to produce protein as the end product.*

*Regulation of gene expression gives control over the timing, location, and amount of a given gene product (protein or ncRNA) present in a cell and can have a profound effect on the cellular structure and function.*

Teat sealing can be one substitute for prophylactic antibiotic dry-cow therapy. Long-lasting, biocompatible preparations can remain stable in the teat canal throughout the dry period and act as a physical barrier against invading pathogens. In fact, previous research found a decrease in infection rate to a third of that of control quarters when quarters were treated with sealant

only during the dry period. In the present experiment, sealant did not affect involution and immune response markers but also did not alter the effect of the chitosan hydrogel. Thus, both approaches are fully compatible and could be used in combination.

## What does it mean?

As concerns around antimicrobial resistance increase, it is essential that the dairy industry explore alternatives to antimicrobials that can be administered at dry-off, which is a particularly susceptible period for high-producing dairy cattle to acquire IMI. Furthermore, it is important to consider the impact on cow welfare resulting from IMI or the general stress of dry-off and the positive effect that prevention of IMI and hastening of the involution process may have.

Although not yet commercially available, compounds such as chitosan hydrogel have the potential to provide a new tool in our toolbox of IMI prevention. The results from this study suggest that a chitosan hydrogel infusion activates immune response and hastens the involution process of the udder. This approach could be used, with or without teat sealant, as an alternative to dry-cow antibiotic therapy for uninfected cows.

## Summary Points

- Preventing intramammary infections at dry-off both improves the welfare of our dairy cows and results in optimal milk production during the next lactation.
- Non-antimicrobial options should be explored for prophylactic use at dry-off, including compounds like chitosan hydrogel.
- Chitosan hydrogel was found to improve immune response and speed up the udder involution process, potentially resulting in fewer intramammary infections during the dry period.
- Teat sealant could be used alongside chitosan hydrogel to further prevent infection by forming a physical barrier.