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Corwin Nelson  
*Iowa State University*

Donald C. Beitz  
*Iowa State University*

John Lippolis  
*United States Department of Agriculture*

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Activation of Vitamin D₃ in Bovine Mastitis Caused by Streptococcus uberis

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Corwin Nelson, graduate research assistant, BBMB; Donald Beitz, distinguished professor of Animal Science, John Lippolis, research molecular biologist, National Animal Disease Center USDA/ARS

Summary and Implications
Inflamed mammary tissue of three cows infected with Streptococcus uberis was found to have higher concentrations of 1α-hydroxylase than un-inflamed control mammary glands. Increased levels of 1α-hydroxylase resulted in increased production of 1,25-dihydroxyvitamin D₃. Therefore, vitamin D₃ may have a role in the inflammation and resolution of bovine mastitis.

Introduction
Streptococcus uberis
S. uberis is among the most common bacterial pathogens that cause clinical mastitis in dairy cows. Intramammary infections caused by S. uberis leads to an inflammatory response that includes increased cytokine expression and infiltration of immune cells. The inflammatory response functions to clear the mammary gland of infection.

Vitamin D
Vitamin D₃ can be acquired in the skin by radiation from UVB light or in the diet and is readily converted to 25(OH)D₃ in the liver. The substrate for 1α-hydroxylase is 25(OH)D₃, which is converted to the active steroid hormone, 1,25(OH)₂D₃. Meanwhile, the vitamin D receptor (VDR) is activated upon binding of the active hormone. Activated VDR functions as a transcription factor by binding vitamin D response elements (VDRE) in promoters of vitamin D responsive genes.

Materials and Methods
Intramammary Infection
Three mid-lactation Holstein cows were infused with 250 CFU of S. uberis in one quarter. The adjacent quarter was infused with phosphate buffered saline as a control. Bacteria were not detected in milk from any of the quarters prior to infection. Seventy-two hours after infection the cows were euthanized and tissue from three separate sites in the control and infected glands was collected.

mRNA Quantification
Total RNA from mammary tissue was isolated and mRNA was reverse transcribed to cDNA. Quantitative real-time PCR using the 2^{ΔΔCt} method was used to measure relative abundance of interleukin 8 (IL-8), 1α-hydroxylase and 24-hydroxylase cDNA. Ribosomal protein S9 (RPS9) was used as the reference gene.

Results and Discussion
Seventy-two hours after infection with S. uberis, all three cows developed clinical signs of mastitis. S. uberis was detected in milk from the infected mammary glands but not in milk from the control glands. Also, IL-8 mRNA was elevated in mammary tissue from the infected gland compared to mammary tissue from the control gland (p < 0.01) (figure 1). IL-8 is a chemokine that is expressed in milk and mammary tissue of inflamed mammary glands and is used here to verify activation of pro-inflammatory genes in the infected mammary glands.

![Figure 1](image_url)

Figure 1. Relative expression of interleukin-8 (IL-8) mRNA in mammary tissue from three separate sites in control and infected mammary glands of three cows at 72 hours after infection with S. uberis. IL-8 was measured by quantitative RT-PCR and normalized to RPS9 gene expression.

The relative amount of 1α-hydroxylase mRNA in control and infected mammary tissue was measured and was found to be much higher in the infected mammary gland (p < 0.01) (figure 2). Production of 1,25(OH)₂D₃ in the infected mammary gland should be higher then since 1α-hydroxylase is the enzyme that converts 25(OH)D₃ to 1,25(OH)₂D₃. A marker of 1,25(OH)₂D₃ production is 24-hydroxylase expression. Expression of 24-hydroxylase is increased by 1,25(OH)₂D₃ via a VDRE in the 24-hydroxylase gene promoter. Therefore, it is shown that production of 1,25(OH)₂D₃ was higher in the infected mammary glands versus the control mammary glands by...
using 24-hydroxylase mRNA as a marker of 1,25(OH)₂D₃ production (p < 0.01) (figure 3).

In conclusion, we have found that vitamin D is activated in inflamed mammary tissue via the expression and activity of 1α-hydroxylase. The definite role of vitamin D in mastitis and the immune response of dairy cattle in general is not yet known. However, vitamin D is known as an anti-inflammatory hormone; so, it may be involved in regulating the inflammatory response in cattle. Vitamin D has also been shown to enhance bactericidal activity in human macrophages; so, it may also be involved in the resolution of bacterial infections in cattle. Therefore, studies regarding the role of vitamin D in mastitis are underway to better understand the fundamental mechanisms of its regulation of the bovine immune system.

**Figure 2.** Relative expression of 1α−hydroxylase (1α-OHase) mRNA in mammary tissue from three separate sites in control λ and infected σ mammary glands of three cows at 72 hours after infection with *S. uberis*. 1α-OHase was measured by quantitative RT-PCR and normalized to RPS9 gene expression.

**Figure 3.** Relative expression of 24-hydroxylase (24-OHase) mRNA in mammary tissue from three separate sites in control λ and infected σ mammary glands of three cows at 72 hours after infection with *S. uberis*. 24-OHase gene expression serves as a marker for 1,25(OH)₂D₃ production in inflamed mammary tissue. 24-OHase was measured by quantitative RT-PCR and normalized to RPS9 gene expression.