Evaluation of Gallium Maltolate on Fecal Shedding of *Salmonella* in Cattle

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**Executive Summary**

**Background**

Foodborne illness is a major cause of morbidity and mortality in the United States. *Salmonella* enterica is the second leading cause of foodborne illness, and causes an estimated 1.4 million cases of gastroenteritis every year. The economic impact of salmonellosis is substantial, with an estimated annual cost of over $3.5 billion due to loss of work, medical care, and life. Salmonellosis is frequently attributed to the consumption of contaminated food products, such as poultry, beef, pork, and eggs. Cattle are a frequent reservoir for *Salmonella*, and novel strategies aimed at minimizing shedding of *Salmonella* are important for reducing the incidence of foodborne illness.

Iron withholding is an essential antimicrobial component of the innate immune system. Iron-binding proteins (particularly transferrin and lactoferrin) bind virtually all free iron in mammalian cells and tissues, thereby rendering iron unavailable to most microorganisms. Some pathogenic bacteria, however, can acquire iron directly from these iron-binding proteins. A variety of preventative and therapeutic strategies for bacterial infections have been developed that act by interfering with microbial iron acquisition and utilization. Gallium is a semi-metal that accumulates in inflamed tissue, macrophages, neutrophils, and in some bacteria. The antimicrobial effects of gallium are related to its ability to be a non-functional iron mimic. Bacteria acquire gallium, instead of iron, and incorporate it into metabolic pathways and enzymes that require iron. Many of these enzymes are critical for survival of the bacteria. The net result is inhibition of bacterial replication, and ultimately bacterial death. Recent publications have shown gallium to exhibit bactericidal activity *in vitro* and *in vivo* against numerous pathogenic bacteria. Preliminary research conducted in our laboratory demonstrated that gallium maltolate, a complex of gallium and maltol with high bioavailability, significantly inhibits growth of *Salmonella enterica* serovar Newport in pure culture and in mixed ruminal fluid cultures.

**Objectives**

The objective of the current project was to determine whether oral administration of gallium maltolate (GaM) would significantly reduce the numbers of *Salmonella* shed in the feces of cattle.

**Methods**

Fourteen five-year-old Holstein steers were housed in a single, outdoor pen with ad libitum access to water, and were group fed at approximately 2.5% body weight (BW) once daily. Steers were randomly assigned to receive either 0 (control), 6.5 g GaM (low dose), or 13.0 g GaM (high dose). Treatment starting dates were staggered by approximately 1 week, such that there were two groups (seven steers/group) in the experiment. Group one consisted of two control animals, three low dose GaM animals, and two high dose GaM animals. Group two consisted of three control animals and four high dose GaM animals.
Both groups were experimentally infected by oral gavage with 550 ml tryptic soy broth containing a cocktail of two \textit{S. enterica} serovars (Newport and Montevideo, herein referred to as \textit{S. newport} and \textit{S. montevideo}). For group one, the first GaM treatment was initiated 60 h after experimental infection. For group two, the first GaM treatment was initiated 24 h after experimental infection.

At the time treatment was initiated, cattle were placed in individual pens and remained there for the duration of the study. Control animals were administered an empty bolus and treatment animals were administered a bolus containing either 6.5 g of GaM, or 13 g of GaM. Fecal samples were obtained every 12 h and processed for qualitative and quantitative analysis of \textit{Salmonella}. Sixty h following the initiation of treatment, animals from both groups were humanely euthanized. Luminal contents and tissue were harvested from the rumen, jejunum, spiral colon, cecum, and rectum. The luminal contents were processed as described above for quantitative and qualitative analysis of the challenge strains of \textit{Salmonella}, and tissue samples were enriched and plated for qualitative analysis of the challenge strains of \textit{Salmonella}.

**Important Results**

The objective of this study was to determine whether oral administration of GaM would reduce fecal shedding of \textit{Salmonella} in cattle over time. Based on preliminary data demonstrating that \textit{in vitro} growth of \textit{S. newport} was significantly reduced in media or rumen fluid containing GaM at concentrations of 250 µM and 500 µM, we used two doses of GaM designed to achieve 250 µM and 500 µM GaM in the rumen. In order to ensure sufficient fecal shedding of \textit{Salmonella}, we challenged the steers with a cocktail of \textit{S. newport} and \textit{S. montevideo}.

We experienced a number of challenges during the execution of this study. In particular, we found that fecal shedding in group 1 appeared to be intermittent, with levels of quantifiable \textit{Salmonella} falling below the limit of detection at some time points, and then rising again at others. We attributed this to the length of time between experimental infection and initiation of treatment, as all animals were consistently shedding quantifiable levels of \textit{Salmonella} for 48 h. Thus, for group two we initiated treatment 24 h following experimental infection (rather than 60 h following experimental infection, as we did for group one), and subsequently analyzed the data separately from group one. Steers from that group shed quantifiable levels of \textit{Salmonella} in their feces throughout the duration of the study. We found no significant differences in fecal shedding between control animals and treated animals in either group 1 (Figure 1) or in group 2 (Figure 2). Similarly, no significant differences were observed when comparing levels of \textit{Salmonella} in luminal contents from control and treated steers or in the frequency of qualitative positive results of luminal contents or tissue samples.
Impact on the Beef Industry

At the present time, the results of this study do not support the use of GaM to reduce fecal shedding of *Salmonella* in cattle. However, these negative findings should be interpreted with caution due to limited statistical power.
Salmonella is a major cause of foodborne illness in humans, and causes over a third of all cases of gastroenteritis in the United States. Human foodborne outbreaks due to Salmonella have been traced to milk, beef, pork, and poultry. Fecal contamination of the carcass and hide is thought to be a major source of tainted meat products derived from cattle. Thus, strategies aimed at reducing fecal shedding of Salmonella and other foodborne pathogens may serve as effective means for limiting transmission of pathogens from food animals to humans. The objective of this study was to determine if oral administration of gallium maltolate (GaM) would reduce fecal shedding of Salmonella in cattle. Gallium is a semi-metal that exhibits antimicrobial properties against some pathogenic bacteria, including Salmonella, by exploiting their requirement for iron to survive and replicate. We administered two doses of GaM to Holstein steers following experimental infection with Salmonella, and monitored quantitative and qualitative fecal shedding in 12 h intervals. Sixty h after initiating treatment cattle were euthanized, and luminal contents and tissue were aseptically harvested from the rumen, jejunum, spiral colon, cecum, and rectum. The luminal contents were processed for quantitative and qualitative analysis of the challenge strains of Salmonella, and tissue samples were enriched and plated for qualitative analysis. We found no significant differences between control animals and treated animals in quantitative levels of Salmonella in the feces or in the luminal contents. Likewise, we observed no pattern between control and treated animals in the frequency of positive or negative values in enriched feces, luminal contents, or tissue samples. Data from this study suggests that GaM was not effective at reducing fecal shedding of Salmonella.

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