Neonatal calf diarrhea: reducing impacts and antibiotic use with lactoferrin and Diaque™

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Introduction

Due to concerns about the human health impacts of agricultural antimicrobial use and antimicrobial resistance (AMR), alternatives to conventional antimicrobials are being sought. As a natural component of colostrum, lactoferrin has antimicrobial and anti-LPS capabilities and may have a role for the treatment of calf diarrhea. Diaque™ (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri) is an oral electrolyte and energy supplement that can be mixed with milk to administer to calves. Diaque may impact the intestinal flora, or may also be a viable option to aid mixture of lactoferrin with milk. This study measured the effects of supplemental lactoferrin and Diaque on concentrations of total and AMR fecal coliforms in two-week-old, Holstein dairy heifers. We hypothesized that supplementation of lactoferrin and/or Diaque would decrease the concentration of total and/or AMR coliforms. Additional objectives of this research included 1) validating a novel technique for measuring AMR coliform concentrations, 2) understanding the dynamics of AMR coliforms early in the calf's life, and 3) determining the association between passive transfer of IgG on shedding of AMR coliforms.

Materials and Methods

The study utilized an experimental field trial that enrolled 44 Holstein heifer calves and randomly assigned them into one of four groups; 2 g Lactoferrin, 500 g Diaque, 2 g Lactoferrin + 500 g Diaque, and no supplements (pasteurized waste milk only). During the calves' second week of life, supplements were provided for two consecutive days, and fecal samples were collected during the two days of supplement administration as well as for two consecutive days following the supplements for a total of four fecal samples from each calf. Ten-fold serial dilutions of each fecal sample were made, and the Colilert® (IDEXX, Westbrook, ME) growth indicator and Quanti-trays® (IDEXX) were used to measure the Most Probable Number (MPN) of total coliforms and coliforms resistant to three different antimicrobials; streptomycin (32 ug/ml), ampicillin (16 ug/ml), and cefotaxime (2 ug/ml). Concentrations of AMR coliforms were estimated by adding a known dilution of antimicrobial to the serially diluted fecal sample and then performing the Quanti-tray® analysis. This Quanti-tray® system was compared to the gold standard, MacConkey agar, for measuring coliform concentrations. Serum IgG and total protein concentrations were also measured for calves during the first week of life to account for failure of passive transfer. Other measurements included calf weights and fecal scores. A random-effects mixed model approach (Proc Mixed, SAS, V.9.3, Cary, NC) was used to compare changes in total and AMR coliforms across each group with a random-intercept for each calf. The Pearson product-correlation was calculated to compare the association of serum IgG concentrations with total and AMR coliform concentrations.

Results

We found that the concentration of total coliforms was significantly higher in calves receiving lactoferrin on the fourth day of the trial ($P < 0.023$); however, neither lactoferrin nor Diaque significantly changed the concentrations of AMR coliforms. Additionally, the proportion of ampicillin and streptomycin resistant coliforms was greater than 95% during the first and second week of life. Overall, age was the greatest predictor of both total and antibiotic resistant coliforms with a significant decrease in concentration by twelve days of age ($P < 0.05$). There was a mild positive association between serum IgG concentrations and concentrations of AMR coliforms for calve in the age range of two to three days ($P = 0.08$).

Significance

Lactoferrin significantly increased the total coliform concentration despite the expected decline. Diaque had no appreciable impact on the concentrations of total or AMR coliforms. Lactoferrin could perhaps have more effects in the small intestine or disrupt the bacterial flora to result in an increase in fecal coliforms. Three other outcomes of the study include; nearly all coliforms were resistant to streptomycin and ampicillin, there was an age-associated decline in AMR coliforms, and serum IgG concentrations were positively correlated with AMR coliform concentrations early in the calves' lives. Ongoing analyses are focusing on the relationship between antimicrobial in the waste milk from treated cows and the proportions of antimicrobial resistance in the calf feces throughout the trial.