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Lactoferrin reduces mortality in preweaned calves with diarrhea

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ABSTRACT

Calf diarrhea is the most common reason for mortality and antimicrobial therapy in preweaned calves on dairy farms in the United States. Conventional and organic livestock producers require alternative therapies for calf diarrhea to reduce the necessity of conventional antimicrobials. Alternatives administered for mild cases or early in the disease course may be useful to mitigate disease progression and reduce the likelihood of septicemia and negative sequelae. Lactoferrin is a bioactive protein naturally found in colostrum that has been shown to prevent septicemia in high-risk infants. Among organic producers, garlic extract is widely used for the treatment of disease and perceived to be efficacious. The objectives of the study were to determine the effectiveness of lactoferrin and garlic extract to reduce mortality and culling, improve weight gain, and reduce the duration of disease in preweaned calves with the first diagnosis of diarrhea. In total, 628 calves with diarrhea from a single commercial dairy were enrolled in a blinded, randomized field trial. Calves diagnosed with diarrhea (fecal score $\geq$3), were randomized to 3 consecutive days of oral garlic extract, lactoferrin, or water (control). Calves were clinically evaluated for up to 10 d. Body weight was measured at enrollment and 10 d later. For calves receiving garlic extract, the risk of death or culling was not significantly different than calves in the control group; however, calves that received lactoferrin had approximately half the risk of death or culling in the 120 d following diagnosis. Additionally, the relative risk of death or culling in the 60 d following diagnosis was significantly lower for the subset of calves with severe diarrhea at enrollment. Neither garlic nor lactoferrin had a significant effect on disease duration or average weight gain during the 10-d period. Lactoferrin significantly reduced mortality and culling when administered to preweaned calves with the first diagnosis of diarrhea; however, additional studies conducted across multiple farms are necessary to corroborate the observed reduction in mortality and culling. If the results are confirmed, lactoferrin may become an important tool to improve treatment outcomes and reduce the necessity of antimicrobials.
Key words: calf diarrhea, lactoferrin, calf mortality, antimicrobial alternative, garlic extract

INTRODUCTION

Epidemiologic linkages between antimicrobial use in livestock and antimicrobial resistance in human pathogens have prompted increasing public and regulatory pressure for reduced livestock antimicrobial use (Dutil, 2010; Saini et al., 2012). However, efforts to restrict antimicrobial use must be balanced against the need to protect livestock health. Alternative therapies are needed to reduce the necessity of conventional antimicrobials important to human health. Additionally, organic livestock producers require alternative therapies to meet stringent USDA requirements that prohibit antimicrobial use within organically certified animals. Avoidance of antimicrobial use is particularly challenging within calf-production systems, where large populations of highly susceptible neonates facilitate rapid transmission of disease. Calf diarrhea is the most common reason for mortality and usage of antimicrobial therapy in preweaned calves on dairy farms in the United States (USDA, 2010; Lombard et al., 2015), and occurs frequently on both conventional and organic farms (Habing et al., 2016). The associated small intestinal overgrowth of *Escherichia coli* and the reasonable likelihood of septicemia in some cases warrants parenteral antimicrobial therapy (Fecteau et al., 1997; Lofstedt et al., 1999; Constable, 2004). However, alternatives to antimicrobial drugs with proven efficacy and administered for mild cases or early in the disease course may be useful to mitigate disease progression, reduce the likelihood of septicemia, and reduce the necessity of antimicrobial use.

Lactoferrin is a bioactive protein naturally found in colostrum that has antimicrobial, antiviral, and immunomodulatory effects (Elass-Rochard et al., 1998; Ellison and Giehl, 1991; Berlutti et al., 2011). It has been extensively investigated as a prophylactic to prevent

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sepsis in high-risk infants (Weinberg, 2001; Zavaleta et al., 2007; Turin et al., 2014). As approximately 30% of calves with severe diarrhea have septicemia (Fecteau et al., 1997; Lofstedt et al., 1999), lactoferrin may also be useful to prevent septicemia when administered to high-risk calves before or early in the course of disease; however, prior studies have evaluated lactoferrin as a supplement, and have either found a positive effect on weight gain or disease prevention (van Leeuwen et al., 2000; Joslin et al., 2002; Robblee et al., 2003; Prenner et al., 2007) or no significant difference (Cowles et al., 2006; English et al., 2007). An experimental challenge trial demonstrated that lactoferrin and lactoperoxidase effectively mitigated the negative health outcomes in calves inoculated with pathogenic *E. coli* (Still et al., 1990). No studies have examined the effect of lactoferrin when administered as a treatment for naturally occurring diarrhea. A randomized clinical trial with naturally occurring cases is necessary to test the effects of lactoferrin as when administered a treatment for calf diarrhea.

Organic producers often rely on natural alternatives to conventional antimicrobials for the treatment of disease, including garlic, aloe, or other herbal therapies (Habing et al., 2016). For instance, approximately 68% of organic producers reported that they used garlic for the treatment of calves with diarrhea, and >75% perceived garlic treatments to be efficacious (Habing et al., 2016). Allicin (garlic) is known to have of in vitro activity against a wide range of gram-negative bacteria, and prior field trials have demonstrated improved growth in calves with dietary garlic extract supplementation (Ghosh et al., 2011, 2010), Therefore, the plausible effectiveness and the widespread use of garlic extract by organic producers warrants a randomized clinical trial to facilitate the creation of evidence-based treatment protocols.

Randomized clinical trials testing novel therapeutics can be used to construct evidence-based treatment protocols for use on the farm. The overall objective of our research was to use a randomized clinical trial to determine the effectiveness of lactoferrin and garlic extract for the treatment of calf diarrhea. We hypothesized that lactoferrin or garlic extract would reduce mortality and culling, improve weight gain, and reduce the duration of disease in preweaned calves with the first diagnosis of diarrhea.

MATERIALS AND METHODS

Setting

The trial was conducted on a single large organic dairy calf ranch in the western United States. All activities were approved by the Ohio State University (**OSU**) Institutional Animal Care and Use Committee (2013A00000054). Holstein calves used in this study were born within a single calving facility on a certified organic dairy operation. Fresh colostrum was harvested at the maternity within 1 to 2 h after calving and all calves received 4 L of optimum quality colostrum (colostrometer; >50 IgG mg/dL) within 4 h after birth either by suckling or via oro-esophageal feeder. Colostrum not immediately fed to calves was refrigerated at 4°C in individual bottles (not pooled) and fed to calves with 2 d of collection. All calves were transported to the calf ranch within 24 h of birth and housed in individual outdoor hutches. For the first 2 feedings, calves were bottle-fed colostrum, and thereafter were bucket-fed twice daily a total of 8 L/d of whole pasteurized milk. A commercial starter grain mix was offered to calves beginning at the third day of life. The targeted weaning age was 56 d, and groups of calves were weaned weekly. At weaning, the milk feedings were reduced to once daily for 1 wk. Weaned calves were moved to group housing and received commercial starter grain for 1 wk, and were then fed a TMR.

Inclusion/Exclusion Criteria

All calves born on the dairy operation and transported to the calf ranch between May 15 and September 16, 2015, were visually assessed in the morning of each day by OSU personnel for the first 3 wk of life. Fecal scores were recorded as a 1 (formed), 2 (semi-formed, pasty), 3 (loose but stays on top of the bedding), or 4 (watery diarrhea that sifts through the bedding). Dehydration scores were recorded as a 1 (no signs of dehydration), 2 (mild depression, skin tent in the neck region of 2–6 s, recessed eyes), 3 (skin tent >6 s, very recessed eyes, and the calf unwilling or reluctant to get up), or 4 (calf will not stand, skin does not flatten when tented; McGuirk, 2008). Calves were enrolled after they were diagnosed by OSU personnel for the first time, with diarrhea (fecal score of 3 or 4). Calves with severe dehydration (dehydration score = 2), with concurrent significant disease (e.g., pneumonia, umbilical infections), or calves that required immediate antimicrobial therapy were excluded. Calves excluded because of pneumonia were those that had an elevated rectal temperature (>39.4 C) with either repeated spontaneous coughs or excessive mucus nasal discharge. Calves excluded due to umbilical disease had an enlarged and painful umbilicus with or without an elevated rectal temperature.

Treatments for calves with diarrhea (e.g., fluids, anti-inflammatories) were administered by farm personnel according to the farm protocols, but study treatments