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Effects of fractionated colostrum replacer and vitamins A, D, and E on haptoglobin and clinical health in neonatal Holstein calves challenged with *Mycobacterium avium* ssp. *paratuberculosis*

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ABSTRACT

Thirty Holstein calves were obtained from 2 dairy farms in central Iowa at birth and randomly assigned to 1 of 6 treatment groups: (1) colostrum deprived (CD), no vitamins; (2) colostrum replacer (CR), no vitamins; (3) CR, vitamin A; (4) CR, vitamin D_3; (5) CR, vitamin E; and (6) CR, vitamins A, D_3, E, with 5 calves per treatment in a 14-d study. Calves were fed pasteurized whole milk (CD) or fractionated colostrum replacer (CR) at birth (d 0) and injected with vitamins according to treatment group. From d 1 through d 14 of the study, all calves were fed pasteurized whole milk (PWM) supplemented with vitamins as assigned. All calves were inoculated with *Mycobacterium avium* ssp. *paratuberculosis* on d 1 and 3 of age. Calves fed CR acquired IgG_1 and haptoglobin in serum within 24 h of birth, whereas CD calves did not. The CR-fed calves were 2.5 times less likely to develop scours, and CR calves supplemented with vitamins D_3 and E also demonstrated a decreased incidence of scours. Serum vitamin levels of A, D, and E increased within treatment group by d 7 and 14 of the study. Interestingly, synergistic effects of supplemental vitamins A, D_3, and E on serum 25-(OH)-vitamin D were observed at d 7, resulting in higher levels than in calves administered vitamin D only. Further, vitamin D_3 deficiency was observed in CD and CR calves fed a basal diet of pasteurized whole milk and no supplemental vitamins. Colonization of tissues with *Mycobacterium avium* ssp. *paratuberculosis* was negligible and was not affected by colostrum feeding or vitamin supplementation. Results demonstrated passive transfer of haptoglobin to neonatal calves, and potential health benefits of supplemental vitamins D_3 and E to calves fed pasteurized whole milk.

Key words: dairy calf, colostrum, vitamin, acute phase protein

INTRODUCTION

Pasteurized whole milk (**PWM**) is a common component in calf diets on commercial dairy farms in the United States, as producers seek to capture losses from waste milk. Recent literature, however, has suggested that PWM alone does not meet the vitamin A, D_3, and E nutritional requirements of young calves and that these requirements are increased by increased growth rates and immune challenge (Krueger et al., 2014; Nonnecke et al., 2014). Suggested mechanisms by which vitamin A and D_3 metabolites affect growth and immunity include the targeting of nuclear receptors to affect cell differentiation, tissue development, and immune cell signaling (Hall et al., 2011; Nelson et al., 2012; Rhinn and Dollé, 2012), whereas vitamin E is an antioxidant that protects cell membrane unsaturated fatty acids from oxidative processes such as respiration and inflammation (Traber and Atkinson, 2007). Pasteurized whole milk contains approximately 38.4 and 1.8 IU/L of vitamins D_3 and E, respectively, falling short of recommended daily allowances (582 and 26.5 IU, respectively) for neonatal calves (NRC, 2001; Krueger et al., 2014). Pasteurized whole milk may satisfy the nutritional requirement for vitamin A in healthy, milk-fed calves, but animals experiencing inflammation have exhibited decreased serum retinol and retinol binding protein (Rosales et al., 1996; Krueger et al., 2014). Thus, supplemental vitamins A, D_3, and E may benefit the neonatal calf. Commercial milk replacers often include vitamins in concentrations above those recommended by NRC (2001) as a recent technical bulletin recommended daily fortification of PWM with 20,000, 7,500, and 100 IU of vitamins A, D_3, and E, respectively, for neonatal calf diets (Wood, 2013).

Although the health of neonatal calves may be affected by vitamin deficiencies, it is clear that colostrum

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intake plays a significant role in immune maturation and decreased morbidity. At birth, newborn calves possess cells and other components that compose the innate and adaptive arms of immunity, but lack endogenous antibody and are highly susceptible to infection at epithelial barriers within the lungs and intestine. Feeding colostrum to calves within the first 24 h of life is accepted widely as an effective means of delivering passive protection in a dose-dependent manner (Robison et al., 1988). Maternal colostrum contains IgG_1 antibodies, leukocytes, complement, and cytokines that confer passive immunity (Chase et al., 2008). Because colostral leukocytes are degraded during the pasteurization and freezing processes that are recommended for commercial handling of colostrum, immune stimulatory benefits are primarily attributed to compounds in the whey fraction. Fractionated colostrum replacer, used in this study, is concentrated colostral whey that contains IgG_1 and many other nonnutritive, bioactive compounds such as IGF, lactoferrin, lysozyme, and lactoperoxidase (Tripathi and Vashishtha, 2006). As passively transferred immunity wanes during early life, acquired immunity is essential for survival, but is potentially impaired by fat-soluble vitamin deficiencies in calves fed only PWM.

Acquired, cell-mediated T_H1 responses, marked by pro-inflammatory cytokines, are an important part of controlling subclinical infections of *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**) in adult bovine animals (Stabel, 2010). Infection rates of this pathogen are greatest during the neonatal stage when fecal-oral transmission risk is high upon exposure to infected dams in the maternity pen. Additionally, transmission to the neonate can occur via colostrum and milk of infected dams (Stabel et al., 2014). Although infection as a neonate is not known to cause acute inflammatory distress, the organism can survive and replicate in the host during a long subclinical latency before overt immune responses become evident (Stabel, 2010). Complications due to colostrum deprivation, vitamin deficiencies, or both may exacerbate the infectivity of young neonates with MAP.

The present study was designed to address the effect of colostrum deprivation and feeding pasteurized whole milk as a basal diet to neonatal calves to determine effects on general health and inflammation. Additionally, supplemental vitamins were provided to provoke calfhood immunity and attenuate inflammatory responses. We sought to use vitamin supplementation standards consistent with commercial standards (Wood, 2013; Krueger et al., 2014), which are in excess of NRC (2001) recommendations. A neonatal MAP infection model was overlaid in the study design to ascertain specific effects of colostrum feeding and vitamin supplementation on uptake of MAP in the first 2 wk of life, a critical period of pathogen susceptibility for the neonatal calf. This study was thus conducted to assess whether colostrum and supplemental vitamins A, D_3, and E affect the inflammatory status of calves fed PWM by measuring serum acute phase proteins haptoglobin (**Hp**) and serum amyloid A (**SAA**), and whether these dietary treatments subsequently alter the MAP infection status of bovine neonates.

MATERIALS AND METHODS

Study Design

Treatments. Thirty Holstein calves were obtained from 2 dairy farms in central Iowa at birth and randomly assigned to 1 of 6 treatment groups: (1) colostrum deprived (**CD**), no vitamins; (2) colostrum replacer (**CR**), no vitamins; (3) colostrum replacer, vitamin A (**CR-A**); (4) colostrum replacer, vitamin D_3 (**CR-D**); (5) colostrum replacer, vitamin E (**CR-E**); (6) colostrum replacer, vitamins A, D_3, and E (**CR-ADE**), with 5 calves per treatment in a 14-d study, as depicted in Figures 1A and 1B. All calves received a first feeding of colostrum replacer or PWM within 4 h of birth; the CD calves were fed 1.9 L of PWM (40°C, Iowa State University Dairy Farm, Ames) as a control, whereas calves in the remaining 5 treatment groups received 375 g of fractionated colostrum replacer (Milk Products, Chilton, WI) reconstituted in 1.9 L of water at approximately 40°C. The colostrum replacer contained 150 g of bovine globulin protein concentrated from colostral whey and was essentially considered devoid of all fat-soluble vitamins A, D_3, and E. Calves received their respective vitamin treatments at the time of first feeding and throughout the study. Calves in vitamin treatment groups were administered 3-mL injections subcutaneously at birth to deliver 150,000 IU of retinyl palmitate (CR-A), 150,000 IU of cholecalciferol (CR-D), and 1,500 IU of D-α-tocopherol (CR-E). Thereafter (d 1–14 of study), calves assigned to vitamin treatment groups were orally administered 25,000, 5,000, and 500 IU of the respective compounds daily in their dietary milk. The CR-ADE calves received an injection or oral solution containing vitamins A, D_3, and E in the concentrations described above. Calves in the CD and CR control groups were injected with 3 mL of placebo carrier solution at birth (proprietary formulation, Stuart Products Inc., Bedford, TX).

Housing and Feeding. All calves were transported within 12 h of birth to the National Animal Disease Center, (**NADC**, Ames, IA). All animal procedures performed were approved by the Animal Care and Use Committee (NADC). Calves were housed indoors in in-