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Effect of feeding whole compared with cell-free colostrum on calf immune status: The neonatal period

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ABSTRACT

Mortality and decreased weight gain resulting from infection and disease in dairy calves are problems within the dairy industry. The bovine neonate relies solely on colostrum to acquire antibodies through passive transfer. To date, colostrum quality is determined by the concentration of antibodies. However, proteins and cells in the colostrum might also enhance immune development in the neonate. To determine the effect of maternal colostral immune cells on calf health and immune status, maternal colostrum was fed either fresh or after lysis of cells by flash-freezing in liquid nitrogen. Thirty-seven female Holstein and Jersey dairy calves were fed 4 quarts total of whole colostrum (WC) or cell-free colostrum (CFC) at birth. Respiratory and fecal scores were measured from birth to d 45 of life. Calf peripheral blood samples were obtained before and after feeding colostrum as well as on d 1, 3, 7, 14, 21, and 28 of life. Peripheral blood mononuclear cells were collected and analyzed for cellular parameters by flow cytometry. Total respiratory scores were greater in CFC-fed calves compared with WC-fed calves on d 38 of life. There were fewer $CD4^+$ T cells and $CD4^+CD62L^+CD45RO^-$ T cells on d 1 and fewer $CD4^+CD62L^+CD45RO^+$ T cells on d 1 and 3 in CFC-fed calves compared with WC-fed calves. Compared with WC-fed calves, CFC-fed calves had a greater percentage of $CD4^+CD62L^-CD45RO^+$ T cells on d 0.25, 1, 3, and 7, and a greater percentage of monocytes on d 7. Our data suggest that colostral cells adoptively transfer and enhance neonatal immunity during the first month of life.

Key words: colostrum, adoptive transfer, immunity, dairy calf

INTRODUCTION

The neonatal immune system is immunologically naïve, making the dairy calf highly susceptible to bacterial and viral pathogens. The National Animal Health Monitoring System reported that 56.5% of all unweaned heifer deaths were due to scours or diarrhea and 22.25% were due to respiratory problems (USDA-APHIS, 2007). Disease incidence in calves negatively affects the profitability of the dairy operation and decreases animal well-being. To abrogate illness and disease in calves, colostrum is required for passive transfer of nutrients and antibodies (Quigley and Drewry, 1998). To date, colostrum quality is determined by antibody content alone. However, colostrum also contains leukocytes, cytokines, antimicrobial proteins, and hormones (Reiter and Brock, 1975; Hagiwara et al., 2000; Blum and Baumrucker, 2002). These colostrum components may have a positive effect on neonatal status and long-term development of immune function.

Similar to passive transfer of antibodies, adoptive transfer delivers colostral leukocytes through the intestinal epithelium and into circulation (Schnorr and Pearson, 1984; Williams, 1993; Liebler-Tenorio et al., 2002). Adoptively transferred colostral cells may be antigen-specific, aiding in immediate pathogen clearance. Antigen-specific colostral cells have memory characteristics and may reside in calf lymphoid tissue until activated by exposure to a future pathogen (Donovan et al., 2007). Previous literature supports a role for maternal colostral cells in modification of adhesion marker expression on T lymphocytes and monocytes. Calves fed cell-free colostrum (**COL−**) had greater numbers and percentages of circulating monocytes expressing CD11a on d 2 and CD11c on d 14 compared with calves fed whole colostrum (**COL+**; Reber et al., 2008a). Calves fed COL− had a greater percentage of T cells expressing CD11a on d 2 and d 14 compared with calves fed COL+. An increase in adhesion markers on immune cells in calves fed COL− may reflect an increase in pathogen insult or stress response compared with calves fed COL+ (Reber et al., 2008a,b).

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The feeding of maternal colostral cells correlates with increased bacterial clearance in the neonate. Riedel-Caspari (1993) experimentally infected calves with *Escherichia coli* and immediately fed either COL+ or COL−. Calves fed COL+ excreted fewer colony-forming units of *E. coli* during the first week of infection. In addition, levels of fecal bacteria in calves fed COL+ reached the lower limit of detectability sooner and concentrations of *E. coli*-specific antibodies during the first 48 h postinfection were higher than in calves fed COL− (Riedel-Caspari, 1993). Similarly, newborn rats fed fresh rat milk, frozen rat milk plus rat milk leukocytes, or commercial formula plus rat milk leukocytes had increased survival following an oral dose of *Klebsiella pneumoniae* compared with rats fed frozen rat milk or commercial formula alone (Pitt et al., 1977). These data suggest a role for adoptively transferred leukocytes through colostrum ingestion on pathogen clearance, neonatal immune function, or both.

In this study, we examined the effect of maternal colostral cells on health and immune cell profiles in dairy calves. We hypothesized that feeding whole colostrum (**WC**) containing intact, viable cells at birth would enhance levels of cellular blood parameters within the first month of life compared with feeding cell-free colostrum (**CFC**) in which cells were lysed.

MATERIALS AND METHODS

Feeding Colostrum Containing Intact Versus Lysed Immune Cells

Twenty-nine Holstein and 8 Jersey cows from the Virginia Tech Dairy Center (Blacksburg) were equipped with a birth monitoring system (FoAlert, Acworth, GA). The FoAlert birth monitoring system was used for immediate notification of parturition. In brief, a veterinarian sutured a transmitter to the outside of the vulva on each cow at ≥2 wk before calving. During parturition, physical separation of the vulva triggered the transmitter, resulting in an audible alarm and phone calls to preprogrammed numbers on an automated dialer. This system ensured that staff arrived before parturition and prevented suckling of the dam by the calf. All animal handling and sampling protocols were in accordance with the Virginia Tech's Institutional Animal Care and Use Committee.

After parturition was complete, the calf was cleaned by the cow but not permitted to suckle. The calf was then removed, weighed, and administered one dose of TSV-2 nasal vaccine (Zoetis, Madison, NJ). The dam was moved into a chute where colostrum was collected aseptically from each quarter using a portable milking unit. If clumps or blood was observed in colostrum, mastitis was assumed and the calf was not enrolled. Colostrum was tested with a digital Brix refractometer to assess total colostral protein and samples saved for subsequent standard bacteriology analyses (NMC, 2004). Only quality colostrum with a Brix score of 23 or higher (Deelen et al., 2014) was fed to calves.

Calves were fed quality WC or CFC from their respective dams, according to the following protocols. Following collection and testing, 1.9 L of WC was used to fill two 2-quart bottles. One bottle was fed to the calf within 3 h of birth, and the second bottle was refrigerated (4°C) and, immediately before the second feeding at 5 to 8 h after birth, slowly warmed to 37°C. Refrigeration had no significant effect on cell viability in WC (data not shown).

To lyse immune cells for the CFC treatment, 4 perfluoroalkoxy (**PFA**) bags (Welch Fluorcarbon Inc., Dover, NH) were filled each with 1 L (1.06 quarts) of colostrum per bag. Each PFA bag was placed in a Styrofoam box and covered with liquid nitrogen. Precautions were taken to ensure that liquid nitrogen did not come into direct contact with colostrum. The PFA bag was turned approximately every 3 min until the colostrum was completely frozen. Following complete freezing of colostrum, the first 2 quarts of colostrum were slowly warmed to 37°C and fed to the calf within 3 h of birth. The second 2 quarts of colostrum were thawed at 37°C, transferred to a bottle, refrigerated (4°C), and then slowly warmed to 37°C immediately before the second feeding at 5 to 8 h after birth.

An esophageal tube feeder was used only in cases when calves did not suckle from the bottle. In this study, 5 calves required feeding by esophageal feeder (2 in fresh and 3 in frozen treatment), precluding statistical analyses and any likely effect on our results. Animals fed by esophageal tube feeder received colostrum in the same volume and at the same time points as bottle-fed calves. All calves entered the Virginia Tech dairy herd, and routine farm management protocols were applied.

Blood Collection for Plasma and Isolation of Peripheral Blood Mononuclear Cells

Calves were bled at birth (before colostrum feeding), 6 h after birth (following second feeding of colostrum), and on d 1, 3, 7, 14, 21 and 28. Peripheral blood mononuclear cells (**PBMC**) were isolated on a Ficoll-Paque density gradient (1.077 g/mL; BD Falcon, Franklin Lakes, NJ), from whole blood collected using 40 m*M* EDTA (10% vol/vol) as described previously (Shafer-Weaver et al., 1999). Following initial centrifugation, plasma was removed, aliquoted, and stored at −20°C