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Effects of Intensified Nutrition on Immune Cell Populations in Milk Replacer-Fed Neonatal Calves

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Summary and Implications
Results from the present study confirm the growth-promoting benefits of feeding an intensified milk replacer to dairy calves. Effects of the elevated plane of nutrition on immune variables examined in the present study were minimal. The number of circulating leukocytes and the composition of the PBMC population as well as this population’s general responsiveness and capacity to secrete IgM were not affected by diet. Relative to responses of calves fed the traditional MR, calves fed the intensified MR demonstrated reduced IFN-\(\gamma\) responses and elevated NO responses during the latter stages of the study. Aberrant NO production, however, can result in undesirable host tissue destruction.

Introduction
The neonatal calf has a heightened susceptibility to a variety of infectious diseases. Peripheral blood mononuclear cells (PBMC) from 1-wk-old calves fed colostrum and milk are functionally hyporesponsive when compared to PBMC from adult cattle. The capacity of PBMC from young calves to produce inducible nitric oxide (NO), a component of bactericidal mechanisms of phagocytic leukocytes, and interferon (IFN)-\(\gamma\), a pivotal cytokine in cell-mediated immunity, differ substantially from the capacities of PBMC from adult cattle. In addition, age-related differences in PBMC populations (leukocyte populations from newborn calves are characterized by a higher proportion of \(\gamma\delta\)-T cells) likely contribute to the increased susceptibility of the neonatal calf to infectious disease.

Nutrient requirements of the young calf are not well described. Standard milk replacer formulations and feeding rates as described on feed tags do not provide sufficient nutrients for the calf during certain periods of the year to meet maintenance requirements or to achieve its growth potential. Nutritional insufficiency impacts immune function and infectious disease susceptibility. Conceivably, improved nutrition resulting from the feeding of an intensified milk replacer would promote immune competency at an earlier age.

Based on results from controlled studies and in-house trials demonstrating the beneficial effects of intensified nutrition on growth rate and body composition, several companies are marketing intensified milk replacers. The immunological consequences of feeding intensified milk replacers to neonatal calves, however, have not been investigated. The objective of this study was to compare the composition and functional capacities of PBMC populations from calves fed an intensified milk replacer with PBMC populations from calves fed a diet meeting current industry standards.

Materials and Methods
Calves and Dietary Treatments
Nineteen Holstein bull calves were housed individually in wire pens bedded with straw in a greenhouse facility. All calves were given 3.9 L of pooled colostrum at birth.

Calves were assigned randomly to one of two treatment groups. Treatment (TRT) 1 calves (n = 9) were fed a 20% crude protein, 20% fat industry standard MR (Milk Specialties Co., Dundee IL). In an attempt to simulate typical calf nutrition-management programs, TRT1 calves were offered 1.4% of BW in DM each day or 140% of the recommended feeding rate. Treatment 2 calves (n = 10) were fed a 30% crude protein 20% fat MR (Milk Specialties) offered at 2.5% of BW in DM each day. Calves were fed MR reconstituted to 15% DM twice daily. Starter feed was not offered but fresh water was available at all times.

Blood Collection, and PBMC Isolation, Phenotype and Functional Assays
Peripheral blood was collected biweekly from each calf, starting immediately before initiation of treatments and continuing until 60 d of age. The PBMC used in assays evaluating cell function were isolated and enriched by density gradient centrifugation. Concentrations of IgM and IgG in serum samples were quantified by ELISA. Leukocytes in PBMC populations were phenotyped by flow cytometry. Synthesis of DNA by PBMC was measured by the amount of \([1^H]\)-thymidine incorporated into cells following a 48h incubation. Secretion of polyclonal IgM by PBMC was evaluated in cell cultures incubated for 14 d. The concentration (\(\mu g/ml\)) of IgM in supernatants was quantified by ELISA. Secretion of IFN-\(\gamma\) was evaluated in PBMC cultures incubated for 48 h. The concentration (ng/ml) of IFN-\(\gamma\) in culture supernatants was quantified using an IFN-\(\gamma\) capture ELISA. Production of inducible NO by PBMC was assayed from cultures incubated 48 h.
The amount of nitrite (µM) in 100 µl of culture supernatant was determined by a colorometric assay.

Arithmetic and log10-transformed data were analyzed as a split-plot with repeated measures ANOVA using Statview software (version 5.0, SAS Institute, Inc., Cary, NC). Fisher’s protected-LSD test was applied when significant effects (P < 0.05) were detected by the model.

**Results and Discussion**

**Effect of Intensified Diet on Growth and Health**

Effects of standard and intensified diets on growth performance of calves are shown in **Figure 1**. By 60-d of age, the mean BW of TRT2 calves was 53% greater than that of TRT1 calves (112.9 vs. 73.7 kg). These results are comparable to those of previous studies evaluating effects of nutrient intake on growth performance of Holstein bull calves. The composition of growth caused by feeding intensified milk replacers has been described previously.

Measurement of Ig concentrations in the circulation indicated that the calves (TRT1 and TRT2) received adequate colostrum to assure Ig-dependent passive immunity. Differences in Ig concentrations were not significant at birth or at 12-h of age (data not shown).

**Effect of Intensified Diet on Composition of PBMC population**

Diet did not affect percentages of δγ T cell receptor+, CD4+, and CD8+ T cells, B cells, and monocytes, or the expression of activation markers [i.e. major histocompatibility (MHC) class II antigen and interleukin-2 receptor (IL-2r)] (data not shown).

**Effect of Intensified Diet on PBMC Function**

Diet had no effect (P > 0.05) on mitogen-induced lymphocyte blastogenesis or IgM secretion (data not shown). These data suggest that the standard MR may provide sufficient protein and energy to maintain the general responsiveness of PBMC population and T-dependent B cell responses. Based on these data, functional maturation of the calf’s PBMC population does not appear to be accelerated by an intensified MR.

Secretion of IFN-γ and NO, both essential for effective cell-mediated immunity, were influenced by diet. On d18, pokeweed mitogen-induced IFN-γ responses of TRT2 calves exceeded those of TRT 1 (Figure 2). Responses of TRT2 calves were lower than responses of TRT1 calves on d60.

Nitric oxide secretion by mitogen-stimulated PBMC from TRT1 and 2 calves is shown in **Figure 3**. At 4-d of age, responses of TRT1 and 2 calves to mitogenic stimulation were comparable and substantially less than adult responses. At 18-d of age, NO responses of all calves were comparable to responses of adults. On d60, the PWM-induced NO response of TRT2 calves exceeded responses of TRT1 calves (P < 0.05) and adults (P < 0.05) (Figure 3).
Interferon-γ produced by stimulated T cells and NK cells can lead to the activation of monocytes/macrophages resulting in the release of NO and its derivatives. The production of NO plays a pivotal role in the regulation of physiological processes, host defense, inflammation and immunity. Leukocytes from both TRT1 and TRT2 calves produced substantially less IFN-γ than PBMC from adult cattle. In contrast, NO responses of calf PBMC were comparable to or exceeded responses of PBMC from adult cattle. Although these results are preliminary, they suggest that the plane of nutrition modulates IFN-γ and NO production by PBMC from young calves. Diet-induced alterations of these pivotal functions might influence cell-mediated immune responses.

The elevated NO responses of TRT2 calves relative to responses of adult cattle should be noted given the potential for excess production of NO and NO derivatives (i.e. peroxynitrite and reactive nitrogen intermediates) to cause local and systemic tissue damage. Excess production of NO causes vasodilation, edema, cytotoxicity and mediates cytokine-dependent processes that can cause tissue damage.

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