Nutritional Modulation of the Proliferation and Activation of Blood Lymphocyte Subsets from Milk Replacer-Fed Calves

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Summary and Implications

Feeding greater quantities of protein and energy to neonatal calves was associated with a reduction in proliferative responses of T lymphocyte subsets to in vitro polyclonal stimulation. Feeding an intensified diet was also associated with altered in vitro expression of activation molecules, CD25, CD44, and CD62L. These data suggest that plane of nutrition during the neonatal period influences lymphocyte-activities essential for the development of a normal immune response.

Introduction

The calf has a heightened susceptibility to a variety of infectious diseases during the first weeks of life. The developmental immaturity of the calf's immune system likely contributes to its increased susceptibility to infectious disease. Therefore, new strategies promoting the maturation (i.e. competency) of the calf's immune system during this period of increased susceptibility are needed.

Current recommendations for protein and energy requirements of the young calf are inadequate for optimal growth. Effects of nutrient supply on body composition and performance of neonatal calves are established, however, effects of nutrient supply on immune function in the neonatal calf are not well described. It is widely known that nutritional status influences immune function. Protein-energy malnutrition is associated with depressed humoral and cell-mediated immune (CMI) responses in calves. Over nutrition affects immune function and has recently been associated with immunosuppression.

Activation, differentiation, and trafficking of T cells to sites of infection are essential for an effective immune response. Expression of CD25 on activated T cells, B cells and monocytes allows cell proliferation and differentiation to be driven by the cytokine, interleukin-2 (IL-2). In cattle, activated lymphocytes exhibit increased expression of the IL-2 receptor (CD25). Activated T cells also demonstrate increased expression of the leukocyte adhesion molecule, CD44. This molecule binds to components of the extra-cellular matrix and is essential for movement of T cells through sites of inflammation/infection. Expression of another molecule, CD62L, is required for entry of lymphocytes into lymph nodes. CD62L expression is down regulated after lymphocyte activation. This reduces the clearance of lymphocytes from the circulation and allows their movement to sites of inflammation.

The objective of this study was to evaluate in vitro the proliferative responses and expression of activation antigens (i.e. CD25, CD44, and CD62L) by peripheral blood T lymphocyte populations from calves fed standard and intensified milk replacers.

Materials and Methods

Animals and Treatments

Eight Holstein bull calves and four Holstein juvenile steers (5-6 m of age) were used. Calves were allotted randomly to standard (n = 4) or intensified (n = 4) diets. The standard diet consisted of a 20% CP, 20% F milk replacer (.45 kg/d) and calf starter (offered on restricted basis, 18% CP). The intensified diet consisted a 28% CP, 20% F milk replacer (1.14 kg/d) and calf starter (offered ad libitum, 22% CP).

Flow Cytometric Analysis of Lymphocyte Proliferation and Activation Marker Expression

Blood was collected from calves and steers 7 wk after initiation of dietary treatments. Blood mononuclear cells (PBMC), isolated and enriched by density gradient centrifugation, were stained with PKH67 green fluorescent dye for flow cytometric analysis of lymphocyte subset proliferation. PBMC cultures were nonstimulated (media only), or stimulated with pokeweed mitogen (PWM, 1 μg/mL), and incubated for 6 d at 37°C in a humidified atmosphere with 5% CO_2.

Data were acquired using a BDLSR flow cytometer (Becton Dickinson, San Jose, California). Proliferation profiles were determined for $CD4^+$, $CD8^+$, and $\gamma\delta$ TCR^+ T cell subsets. Data are presented as the mean (± SEM) percent of cells that had proliferated. Proliferation in resting (nonstimulated) cultures was subtracted from that of PWM-stimulated cultures. Expression of CD25, CD44, CD62L data are presented as the mean (± SEM) geometric mean fluorescence intensity (MFI) on $CD4^+$, $CD8^+$, and $\gamma\delta$ TCR^+ cells.

Statistical Analysis

Growth data were analyzed with repeated measures ANOVA (Statview 5.0 SAS Institute, Inc., Cary, NC). Activation data were analyzed as a split-plot, factorial

ANOVA. Fisher's protected-LSD test was applied when significant effects were detected ($P < 0.05$).

Results and Discussion

Effect of Intensified Nutrition on Growth Performance

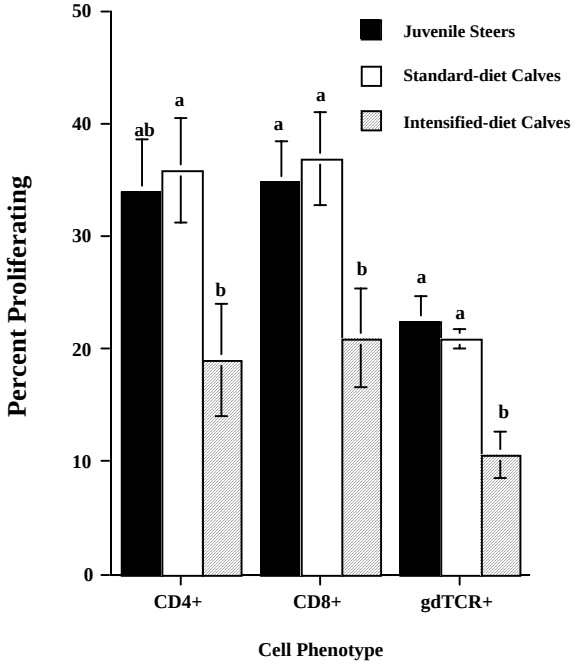
Body weights of standard-diet calves (n = 4) and intensified-diet calves (n = 4) were not different at the beginning of the trial. Mean body weight of intensified-diet calves was greater than mean body weight of standard-diet calves from wk 2 to 7 of the experimental period. The average daily weight-gain of intensified-diet calves (.66 kg/d) exceeded that of standard-diet calves (.27 kg/d) during the experimental period.

In Vitro Proliferative Responses of Lymphocytes 7 wk after Initiation of Dietary Treatments

Pokeweed mitogen-induced proliferative responses of lymphocytes from calves 7 wk following initiation of dietary treatments (8 wk of age) are shown in Figure 1. Percentages of $CD4^+$, $CD8^+$ and $\gamma\delta TCR^+$ cells proliferating in response to PWM were not different between standard-diet calves and steers. Percentages of $CD8^+$ and $\gamma\delta TCR^+$ cells proliferating in response to PWM, however, were greater in steers than in intensified-diet calves.

Dietary treatment affected proliferative responses to PWM. Percentages of $CD4^+$, $CD8^+$ and $\gamma\delta TCR^+$ cells proliferating in response to PWM were greater in standard-diet calves than in intensified-diet calves.

Figure 1. Mitogen induced proliferative responses of $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ from standard- and intensified-diet calves, and steers.



In Vitro Expression of CD25, CD44, and CD62L on Lymphocytes 7 wk Following Initiation of Dietary Treatments

PWM-induced expression of CD25, CD44, and CD62L on lymphocyte subsets from calves and steers is shown in Tables 1-3.

CD25 expression on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from standard-diet calves was comparable to that of steers and increased in PWM-stimulated cultures compared with nonstimulated cultures (Table 1). Similarly, expression of CD25 on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from intensified-diet calves was increased in cultures stimulated for 6 d when compared to nonstimulated cultures (Table 1). Standard-diet calves had increased expression of CD25 on $CD8^+$ cells compared with intensified-diet calves, suggesting that plane of nutrition affects CD25 expression on activated $CD8^+$ cells.

Expression of CD44 on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from standard-diet calves was comparable to that of steers. CD44 expression on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from standard-diet calves, intensified-diet calves, and juvenile steers was increased in PWM-stimulated cultures compared with nonstimulated cultures (Table 2). Intensified-diet calves had significantly lower expression of CD44 on PWM-stimulated $\gamma\delta TCR^+$ cells compared with juvenile steers (Table 2). These data suggest than an increased plane of nutrition provided by an intensified milk replacer may decrease expression of a cell surface molecule that plays a critical role in the migration of lymphocytes through infected tissues.

Expression of CD62L on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from standard-diet calves was decreased in PWM-stimulated cultures when compared with nonstimulated cultures (Table 3). CD62L expression on $\gamma\delta TCR^+$ cells from juvenile steers and intensified-diet calves was not affected by stimulation (Table 3). Steers had increased expression of CD62L on $\gamma\delta TCR^+$ cells after 6 d of PWM stimulation when compared with intensified-fed calves (Table 3).

These data indicate that the increased plane of nutrition provided by an intensified milk replacer influences both the proliferative responses and expression of activation markers by T cells. Taken together, these results suggest T cells from calves fed intensified milk replacer were less responsive to polyclonal stimulation than were T cells from calves fed a standard milk replacer. Interestingly, responses of cells from calves fed standard milk replacer were more similar to responses of cells from the older steers than to responses of cells from intensified-fed calves. Additional research is necessary to determine whether these nutrition dependent differences in T cell responsiveness affect the susceptibility of the calf to infectious disease.

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Table 1. CD25 mean fluorescence intensity on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ T cells from juvenile steers and calves 7 wk after initiation of dietary treatments.

Cell Phenotype	Non-stimulated	PWM	Mitogen Effect
CD4+			
Juvenile	391 ± 97[b]	1567 ± 207	<0.001
Standard	776 ± 134[a]	1658 ± 120	<0.01
Intensified	662 ± 125[ab]	1330 ± 174	<0.01
CD8+			
Juvenile	46 ± 6	1065 ± 129[a]	<0.0001
Standard	85 ± 19	1193.5 ± 270[a]	<0.001
Intensified	128 ± 8	828.5 ± 128[b]	<0.001
γδTCR+			
Juvenile	40 ± 3[b]	928 ± 224	<0.001
Standard	69 ± 17[ab]	626 ± 87	<0.0001
Intensified	94.7 ± 8[a]	495.5 ± 80	<0.001

[a,b] Values with differing superscripts within a cell phenotype within a column are different ($P < 0.05$).

Table 2. CD44 mean fluorescence intensity on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ T cells from juvenile steers and calves 7 wk after initiation of dietary treatments.

Cell Phenotype	Non-stimulated	PWM	Mitogen Effect
$CD4^+$			
Juvenile	1033 ± 165[b]	3059 ± 87	<0.0001
Standard	2009 ± 206[a]	3071 ± 195	<0.01
Intensified	1824 ± 192[a]	2665 ± 108	<0.05
$CD8^+$			
Juvenile	626 ± 80	2716 ± 443	<0.001
Standard	924 ± 240	2756 ± 246	<0.001
Intensified	1040 ± 88	1982 ± 139	<0.001
$\gamma\delta TCR^+$			
Juvenile	402 ± 28	2310 ± 433[a]	<0.001
Standard	671 ± 118	1616 ± 70[ab]	<0.001
Intensified	588 ± 90	1315 ± 177[b]	<0.01

[a,b] Values with differing superscripts within a cell phenotype within a column are different ($P < 0.05$).

Table 3. CD62L mean fluorescence intensity on $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells from juvenile steers and calves 7 wk after initiation of dietary treatments.

	Non-stimulated	PWM	Mitogen Effect
CD4+			
Juvenile	868 ± 151	134 ± 23	<0.05
Standard	1375 ± 277	122 ± 17	<0.01
Intensified	836 ± 186	129 ± 54	<0.01
$CD8^+$			
Juvenile	920 ± 172[b]	304 ± 26	<0.05
Standard	2046 ± 543[a]	257 ± 73	<0.01
Intensified	446 ± 129[b]	158 ± 15	<0.05
$\gamma\delta TCR^+$			
Juvenile	566 ± 159[ab]	338 ± 17[a]	NS
Standard	1376 ± 404[a]	271 ± 22[ab]	<0.01
Intensified	276 ± 80[b]	222 ± 33[b]	NS

[a,b] Values with differing superscripts within a cell phenotype within a column are different ($P < 0.05$).