High-Growth Rate Fails to Enhance Adaptive Immune Responses in Neonatal Calves and Decreases Immune Cell Viability

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Monica R. Foote, graduate research assistant; Brian J. Nonnecke, lead scientist, National Animal Disease Center; W. Raymond Waters, scientist, National Animal Disease Center; Donald C. Beitz, distinguished professor of animal science and biochemistry, biophysics, and molecular biology

Summary and Implications

The objective of the current study was to investigate the effects of different feeding rates achieving three targeted growth rates (No Growth, Low Growth, and High Growth) on adaptive immune responses of neonatal calves vaccinated with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) and ovalbumin (OVA) 3 wks after initiation of dietary treatments. The daily growth rates for No-, Low-, and High-growth calves were different throughout the experimental period and averaged 0.11 ± 0.02 kg, $0.58 \pm .02$, and 1.16 ± 0.04 kg, respectively. Adaptive immune responses generally were not affected by growth rate. Ovalbumin-specific IgG_1 and IgG_2 concentrations after vaccination were not affected by growth rate. Interferon (IFN)-γ and nitric oxide (NO) secretion by PPD-stimulated mononuclear leukocytes (MNL) also were not affected by growth rate. Antigen (i.e., PPD)-elicited delayed-type hypersensitivity in No-growth calves was greater than Low-growth but similar to High-growth calves. Viability of MNL, $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells in stimulated and non-stimulated cultures from High-growth calves was substantially lower compared with No- and Low-growth calves. These results suggest protein-energy malnutrition (PEM) in the absence of weight loss does not affect negatively adaptive immune responses of calves and that increasing growth rate or plane of nutrition above maintenance requirements does not benefit adaptive immune responses. High rates of growth, however, may affect negatively immune cell viability, with potentially deleterious effects on the calf's resistance to infectious disease.

Introduction

Neonatal animals are highly susceptible to bacterial and viral pathogens. Traditional calf-rearing programs limit nutrient intake from milk or milk replacer during the first few weeks of life in order to promote dry feed (i.e., starter) intake and allow early weaning. Recent reports of dramatic improvements in the growth performance and feed efficiency resulting from feeding greater amounts of milk replacer with higher protein concentrations has led to interest in intensified or accelerated feeding programs. It is believed that intensified feeding programs increase the plane of nutrition to more "natural" levels and provide more "biologically appropriate" early growth. Improving the plane of nutrition also may improve calf health and decrease morbidity and mortality.

Protein-energy malnutrition is the major cause of immunodeficiency worldwide. Protein-energy malnutrition manifests as acute (wasting) and chronic (stunting) forms, resulting in altered body composition and decreased linear growth, respectively. Reports suggest that stunting and wasting PEM may depress cell-mediated immunocompetence and increase the risk of infection-related mortality. Most experiments to date have investigated effects of wasting-, not stunting-, PEM on immunity. Substantial evidence supports negative effects of PEM on adaptive immune responses. Alternatively, restricting dietary protein and energy affects positively immune function and longevity in rodents.

We hypothesize that increasing plane of nutrition above maintenance requirements does not enhance adaptive immune responses in vaccinated neonatal calves.

Materials and Methods

Animals

Twenty-four Holstein bull calves were acquired from a single Wisconsin dairy herd over a 2-wk period. All calves were given 3.9 L each of colostrum within 6 h of birth. After birth, calves were transported to the National Animal Disease Center, ARS, USDA, Ames, IA, where they were housed individually in elevated pens in a temperature-controlled (64°C) barn.

Dietary Treatments

Treatments were designed to achieve three targeted daily rates of gain (No Growth = 0.0 kg, Low Growth = 0.55 kg, or High Growth = 1.2 kg) in live weight over a 7-wk period. The NRC Nutrient Requirements of Dairy Cattle calf model computer program was used to estimate milk replacer intakes needed to achieve target growth rates. All calves were fed a 30% CP, 20% F all-milk protein milk replacer (Land O' Lakes, Inc.) reconstituted to 14% dry matter. Calves were weighed each Monday at midday, and diets were reformulated to allow for changes in live weight. No starter grain was offered, and water was offered *ad libitum*.

Vaccination

At wk 3 of the experiment, all calves were vaccinated subcutaneously in the right midcervical region with 10^7 cfu of *M. bovis* BCG. At wk 3 and wk 5 of the experiment, adjuvanted OVA (4 mg OVA in incomplete Freund's adjuvant) was administered to all calves subcutaneously in the left midcervical region.

Blood Collection

Peripheral blood (90 mL) was collected via jugular venipuncture at wk 0, 3 (time of primary sensitization), 5 (time of OVA secondary vaccination), 6, and 7 of the experimental period. Coagulated (no additive) blood samples also were collected once weekly during the experimental period for quantification of antigen-specific IgG concentrations.

Recall Antigens

Recall antigens used for *in vivo* and *in vitro* assays were crystallized ovalbumin (Grade V, Sigma) and *M. bovis*-derived purified protein derivative (PPD; Pfizer, Kalamazoo, MI.).

Assays

OVA-specific IgG_1 and IgG_2 concentrations in serum samples collected weekly before and after vaccination were determined by a capture ELISA.

Cells used in IFN-γ assays were from blood samples collected at wk 3, 5, and 7 of the experimental period. Wells were seeded with 4×10^5 cells in a total culture volume of 200 μL. Cultures were either non-stimulated (medium alone) or stimulated with PPD (10 μg/mL) or OVA (10 μg/mL). Cultures were incubated at 39°C in a humidified atmosphere of 5% CO_2 for 72 h. Supernatants were subsequently harvested from centrifuged plates and stored at -80°C. The IFN-γ concentration (ng/mL) in culture supernatants was determined by using an IFN-γ capture ELISA.

Cells used in NO assays were from blood samples collected at wk 3 (before vaccination), 5, and 7 of the experimental period. Wells were seeded with 4×10^5 cells in a total culture volume of 200 μL. Cultures were non-stimulated (medium alone), stimulated with 10 μg/mL OVA, or with 10 μg/mL *M. bovis* PPD. Plates were incubated at 39°C in a humidified atmosphere of 5% CO_2 for 48 h. The amount of nitrite in culture supernatants was measured using the Griess reagent.

In vivo sensitization to *M. bovis* BCG was evaluated by using the comparative cervical skin test following Bovine Tuberculosis Eradication Uniform Methods and Rules (Animal and Plant Health Inspection Service brochure #91-45-011). Skin-fold thickness was measured immediately before administration of PPD and 72 h later.

Viability of MNL

Viability of blood MNL in non-stimulated and OVA- and PPD-stimulated cultures was evaluated when calves had been on dietary treatments for 7 wk. Cultures were non-stimulated (media only) or stimulated with OVA (10 μg/mL) or PPD (10 μg/mL) and incubated for 3 d at 39°C in a humidified atmosphere with 5% CO_2. Cells were labeled with one of three phenotype makers (CD4, CD8, or γδTCR) and with 7-amino actinomycin D (7-AAD). Cells were analyzed via flow cytometry. Cells without 7-AAD labeling were considered viable, whereas apoptotic and dead cells showed low and high 7-AAD staining, respectively.

Results and Discussion

Growth Performance

This study describes, for the first time, the effects of feeding at different rates to achieve targeted daily growth rates on adaptive immune responses in neonates. Mean body weights of calves from the three treatment groups were different ($P < 0.05$) by wk 1 (Figure 1). The daily growth rates for No-, Low-, and High-Growth calves were different ($P < 0.0001$) throughout the experimental period and averaged 0.11 ± 0.02 kg, $0.58 \pm .02$, and 1.16 ± 0.04 kg, respectively.

Antibody Response to Ovalbumin Vaccination

Serum OVA-specific IgG_1 and IgG_2 increased ($P < 0.05$) following vaccination (Figures 2a and 2b, respectively). Growth rate did not affect ($P > 0.5$) OVA-specific IgG_1 or IgG_2 concentration in serum across time, suggesting calves fed a diet resulting in no growth have normal in vivo antibody responses. Two wk following primary vaccination (wk 5), concentration of OVA-specific IgG_2 was higher ($P < 0.05$) in High-Growth than in Low-Growth calves. Concentration of OVA-specific IgG_2 was similar ($P > 0.05$) in High- and No-Growth calves.

Secretion of IFN-γ and NO by Blood MNL in Response to Antigen

We have shown previously that *M. bovis* BCG-vaccinated calves exhibit strong cell-mediated immune (CMI) responses to PPD. In the present study, a BCG sensitization and challenge model was used to evaluate effects of growth rate on adaptive CMI responses of milk replacer-fed calves. By using this model, we evaluated IFN-γ and NO production in antigen-stimulated blood MNL cultures, which are functions intimately associated with CMI. Effects of growth rate on secretion of IFN-γ in OVA- and PPD-stimulated cultures are shown in Figure 3. Secretion of IFN-γ by OVA-stimulated blood MNL from No- and High-Growth calves increased ($P < 0.01$) with time following vaccination, whereas IFN-γ responses to OVA stimulation by MNL from Low-Growth calves did not change ($P > 0.05$) with time (Figure 3a). Growth rate did not affect ($P > 0.05$) secretion of IFN-γ by PPD-stimulated blood MNL from calves (Figure 3b). Secretion of IFN-γ by

PPD-stimulated MNL did increase ($P < 0.01$) with time following vaccination.

Effects of growth rate on NO production are shown in Figure 4. Growth rate did not affect ($P > 0.05$) NO production by PPD-stimulated MNL. Production of NO by PPD-stimulated blood MNL from High-, Low-, and NO-Growth calves increased ($P < 0.05$) with time following vaccination.

Cutaneous delayed-type hypersensitivity (DTH)

Responses to intradermal administration of *M. bovis* PPD 5 wk following vaccination are shown in Figure 5. Change in skin fold thickness was greater ($P < 0.05$) in No-Growth calves compared with Low-Growth but not High-Growth calves, suggesting that calves fed a diet resulting in no growth have normal DTH reactions that are indicative of competent *in vivo* CMI responses.

Blood MNL viability in culture

Growth rate affected the viability of blood MNL in non-stimulated and antigen-stimulated cultures (Figure 6). Percentages of viable MNL, CD4$^+$, CD8$^+$, and γδTCR$^+$ cells in non-stimulated (Figure 6a) and PPD-stimulated cultures (Figure 6b) established from cells isolated from High-Growth calves were lower ($P < 0.01$) compared with No- and Low-Growth calves.

Conclusions

The effects of growth rate on adaptive immune responses to OVA and *M. bovis* BCG vaccination in calves were minimal, suggesting PEM in the absence of weight loss does not affect adaptive immune responses of the neonate. Weight loss may be a requirement to achieve PEM-induced immunodeficiency. High growth rates, however, decreased immune cell viability, having potentially deleterious effects on disease resistance. Further investigation is required to determine if high rates of growth induce metabolic/oxidative stress on cells of the immune system.
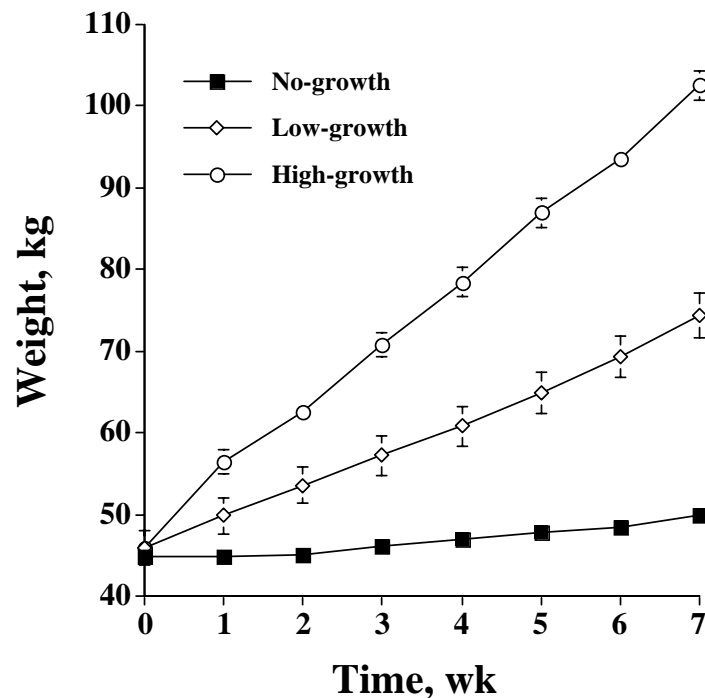
Figure 1. Growth performance of calves fed milk replacer at three rates of intake to achieve three targeted daily rates of gain.
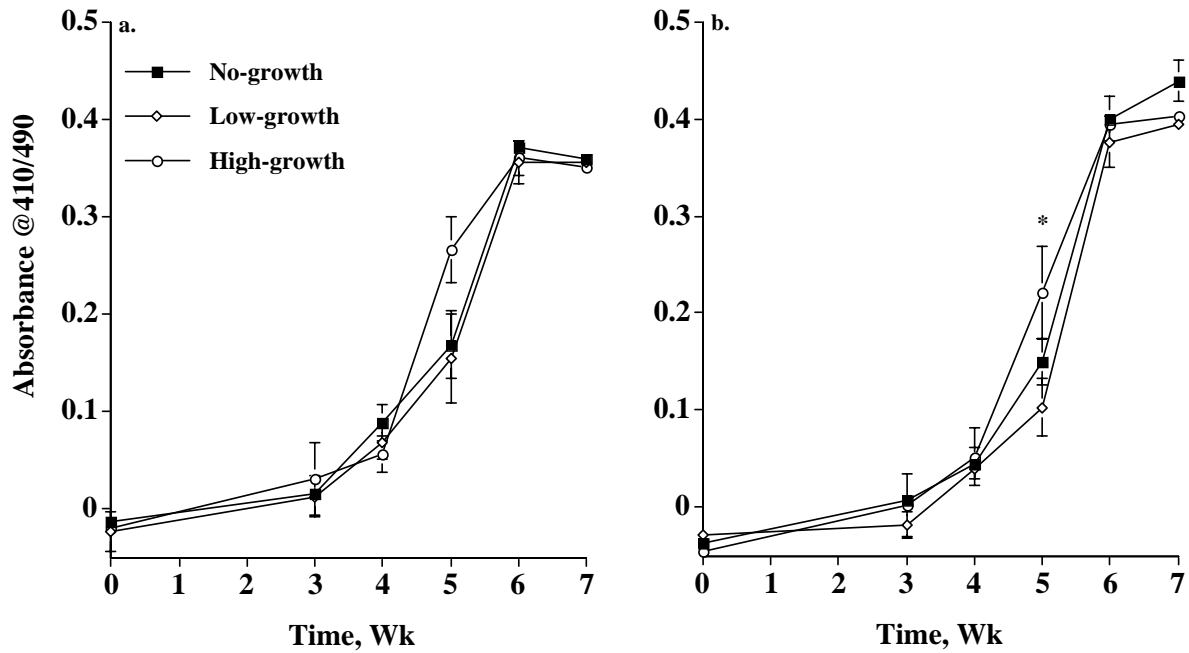
Figure 2. Relative amounts of ovalbumin-specific IgG$_1$ (panel a) and IgG$_2$ (panel b) in serum from calves fed milk replacer at three rates of intake to achieve no (n = 8), low (n = 8), or high (n = 8) growth rates.
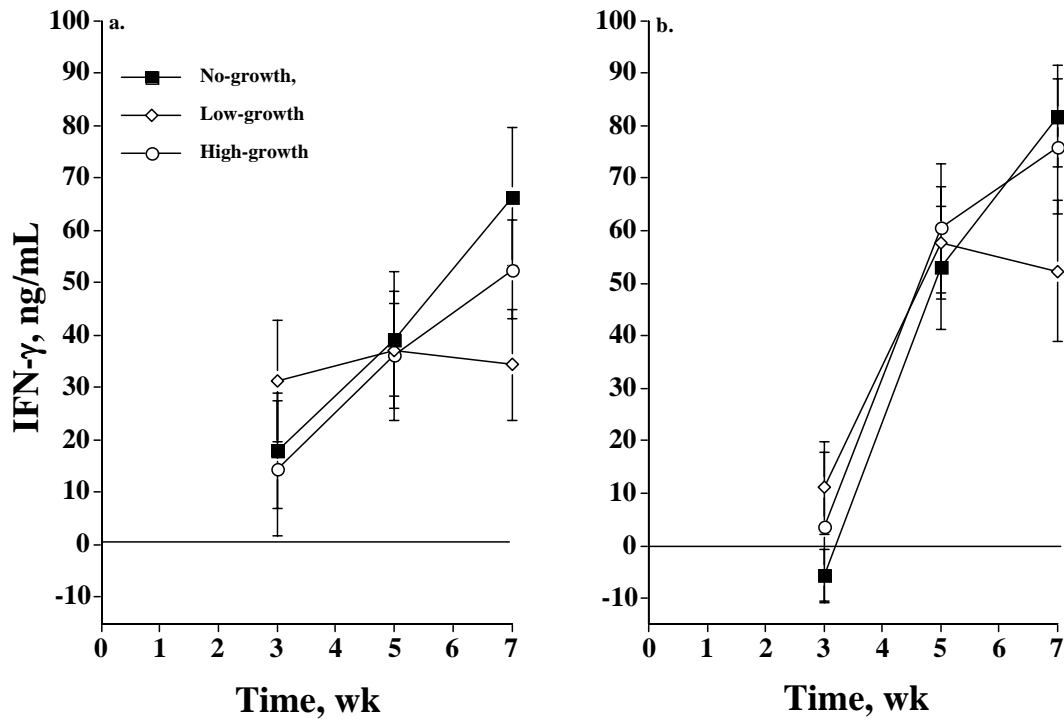

Figure 3. In vitro interferon (IFN)-γ secretion by blood mononuclear cells (MNL) from calves fed milk replacer at three rates of intake to achieve no (n = 8), low (n = 8), or high (n = 8) growth rates.
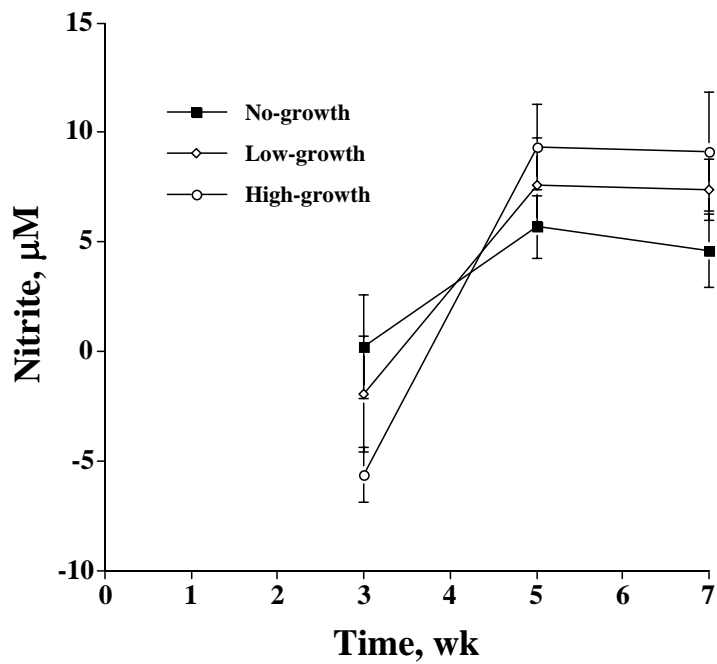
Figure 4. In vitro nitric oxide (NO) secretion by blood mononuclear cells (MNL) from calves fed milk replacer at three rates of intake to achieve no (n = 8), low (n = 8), or high (n = 8) growth rates.
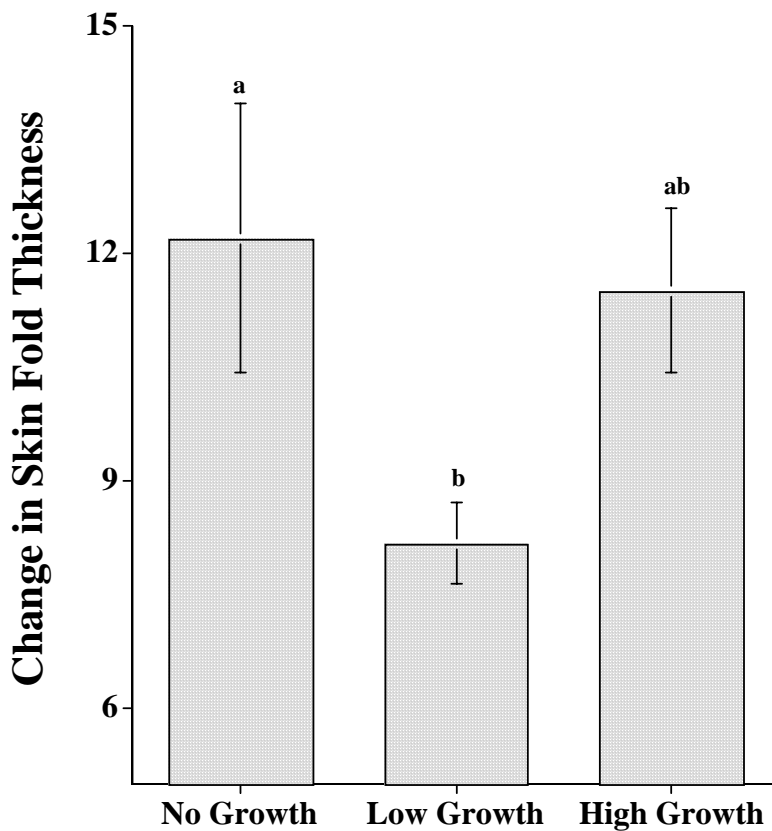

Figure 5. *In vivo* delayed-type hypersensitivity reactions of calves fed milk replacer at three rates of intake to achieve no (n = 8), low (n = 8), or high (n = 8) growth rates.
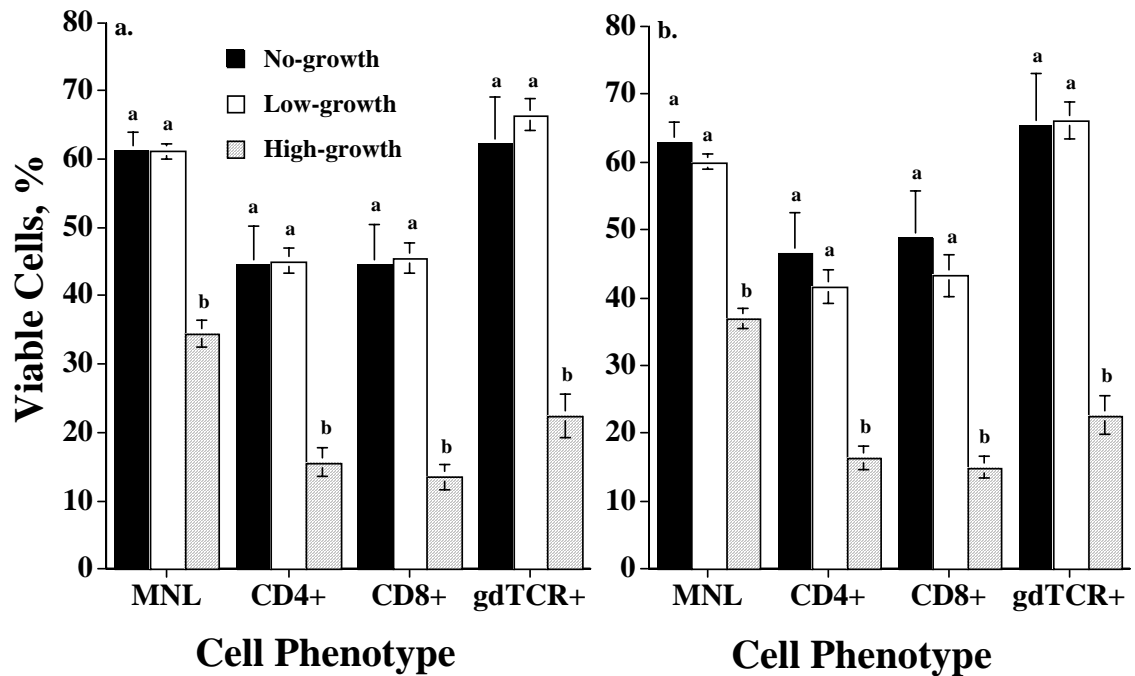
Figure 6. Viability of blood mononuclear cells (MNL) from calves fed milk replacer at three rates of intake to achieve no (n = 4), low (n = 4), or high (n = 4) growth rates for 7 wk.