INTENSIFIED NUTRITION AND THE ADAPTIVE IMMUNE RESPONSE OF THE PREWEANED CALF

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During the first weeks of life, the calf experiences a heightened susceptibility to a variety of infectious diseases. Developmental immaturity of the neonatal immune system is considered a significant contributory factor to the newborn's susceptibility to bacterial, viral, and protozoan infections. Based on National Health Monitoring Service (NAHMS) data from 1996 and 2002, the mortality rate for pre-weaned calves is approximately 8 to 11%. The NAHMS (1994) survey data also indicate that morbidity rate for preweaned calves is approximately 37%. Taken together, these data imply that more than 40-45% of calves are either ill or die before weaning. Although on-farm costs associated with calfhood infectious disease are substantial (annual costs estimated to be between $90–180 million dollars and approximately 1.3 million man-hours), these diseases also impact human health and food-safety through pre-harvest contamination and zoonotic transmission.

Nutritional factors (i.e. plane of nutrition) may have a profound influence on the development (i.e. maturation) of the immune system and consequent resistance of the preweaned calf to infectious disease. Optimizing immune competency using nutritional approaches not only has the potential to increase infectious disease resistance but also lessen the need for antibiotics, and limit antibiotic and pathogen contamination of meat and milk. And finally, optimal nutrition during the neonatal period may promote development of a more immunologically robust adult.

This paper presents preliminary data from controlled experiments examining effects of intensified nutrition programs on the adaptive immune response (**IR**) of the milk replacer-fed dairy calf.

Intensified Nutrition Programs

Calf-rearing programs traditionally have limited nutrient intake from milk or milk replacer during the first few weeks of life in an attempt to promote dry feed intake (i.e. calf starter) and allow early weaning. Standard milk replacer formulations and feeding rates as described on feed tags may not provide sufficient nutrients for the calf to achieve its growth potential or during certain times of the year (i.e. winter) to meet maintenance requirements. Several studies have established that feeding greater amounts of milk replacer with higher protein concentrations dramatically improves the growth performance and feed efficiency of the preweaned calf (Bartlett 2001, Blome 2002, Diaz et al., 2001, Tikofsky et al., 2001). As noted in a recent review of the subject (Drackley, 2005), these research results have been the impetus for the development and commercialization of intensified nutrition programs that elevate the plane of nutrition to more "natural" levels and provide more "biologically relevant" early growth. Several companies now market intensified milk replacers (e.g. Advance Excelerate® Calf Milk Replacer, Milk Specialties, Dundee, IL; Cow's Match®, Land O'Lakes, Inc., Milk Products Co., Fort Dodge, IA; and Super Star 25-15®, Merrick's Inc., Middelton, WI). The success of "intensified nutrition" programs is dependent on several outcomes that include shortened time to first calving and heightened production capacity (i.e. milk production) as adults (Drackley, 2005). These outcomes are necessary to justify the costs, increased intensity of management, and delayed rumen development associated with the implementation of intensified, calf-feeding programs. Conceivably, intensified nutrition programs may enhance immune function resulting in decreased morbidity and mortality associated with calfhood, infectious diseases.

Brief Overview of the Immune System of the Neonatal Calf

Under normal circumstances, the calf's exposure to infectious agents/antigens in utero is rare. Immediately after birth the calf is exposed to large numbers of organisms and resistance to infection depends both on the protective factors present in colostrum and on its inherent capacity to develop innate (i.e. not antigen specific but dependent on macrophages and neutrophils) and adaptive (antigen-specific) IR. A general assumption is that the immune system of the neonate is immature and incapable of mounting adult-like responses to antigenic stimulation (Adkins, 2000, Morein et al., 2002). Developmental immaturity of the

calf's immune system may compromise the efficacy of vaccination protocols and exacerbate the calf's susceptibility to infectious disease. The adaptive immune system of the neonate is characterized by a T-cell population with a high proportion of naïve cells that can suppress immunoglobulin (**Ig**) production (Clement et al., 1990). Neonates also have higher proportions of antigen-presenting cells with defective co-stimulatory activity (Ridge et al., 1996) and a decreased capacity to produce cytokines; particularly those associated with cell mediated [i.e. $T_{helper}1$ (**Th1**)] immune (**CMI**) responses (Adkins, 2000, Siegrist, 2000). The Th1-biased response is necessary for protection against viruses and intracellular bacteria and is characterized by the predominant production of interferon (**IFN**)-γ. In infant mice, exposure to antigen leads to a $T_{helper}2$ (**Th2**) biased response characterized by interleukin (**IL**)-4 secretion and antibody responses predominated by the IgG_1 isotype. The ruminant animal is also unique in that it is born agammaglobulinemic (i.e. no serum Ig) and requires high quality colostrum for acquisition of maternal Ig and possibly viable leukocytes for early protection against infection (Barrington and Parrish, 2001, Kumar et al., 1989).

Development of the adaptive (antigen-driven) IR has not been characterized extensively in preweaned dairy calves. It is generally assumed that the calf is incapable of mounting an effective adaptive IR during the first weeks of life. To test this assumption, we evaluated the effects of early vaccination (at 1 wk-age) on the adaptive IR of calves and compared these responses to those of adult cattle vaccinated in an identical fashion (Nonnecke et al., 2005). Calves vaccinated with an attenuated strain of *Mycobacterium bovis* [strain bacillus Calmette Guerin (**BCG**)] during the first week of life produced Th1-like responses to antigen (i.e. **PPD**, purified protein derivative) that were comparable with antigen elicited responses of vaccinated adult cattle. Development of a CMI response [i.e. increased IFN-γ and inducible nitric oxide (**NO**)] was evident in vaccinated calves and adults by wk 2 post-vaccination and persisted for the duration of the 11 wk study. Although vaccinated adults developed measurable antibody responses, responses of vaccinated calves were undetectable. Reasons for the inability of vaccinated calves to mount demonstrable antibody responses to BCG vaccination were not determined; however, maternal cross-reactive antibody was likely a factor. Colostral antibodies, although important for protection of the neonate, can inhibit antibody responses to vaccination/infection (Barrington and Parrish, 2001, Endsley et al., 2003).

For the animal scientist studying effects of nutritional status, individual micronutrients, or endocrines on the calf's immune system, the BCG sensitization/PPD challenge model has several appealing characteristics. Because BCG is attenuated, vaccination is not associated with horizontal transmission of

the organism. Secondly, PPD-elicited recall responses in cattle have been described allowing effects of potential modulators of the IR to be analyzed in detail. We also have evaluated a second sensitization system. Vaccination of neonatal calves with ovalbumin **(OVA)**, an antigen not present in the natural environment of dairy cattle, elicits measurable antibody responses that are amplified when calves are revaccinated before weaning [unpublished data, Fig 9 (left panel)]. These sensitization protocols have provided data indicating that the calf is capable of mounting robust, antigen-dependent CMI and antibody responses when vaccinated early in life. Demonstration of a functional adaptive IR by the newborn calf provides a valuable tool for examining the effects of neonatal nutrition on immune responsiveness.

Results from Recent Experiments Evaluating Immunological Consequences of Intensified Nutrition

In a series of collaborative studies, we have begun to compare immune function parameters in preweaned calves fed standard and intensified milk replacers. The underlying hypothesis of this research is that increasing the plane of nutrition during the first weeks of postnatal life enhances the functional capacity of the adaptive arm of the calf's immune system resulting in a concomitant increase in infectious disease resistance. Holstein bull calves used in these studies all received high quality colostrum to assure acquisition of passive immunity. Calves were 4 to 8 d of age at the beginning of study. In all trials, growth rates of calves fed intensified milk replacers exceeded the growth rates of calves fed industry-standard milk replacers. Health status of calves fed standard and intensified milk replacers was comparable in all trials.

In the first trial (Nonnecke et al., 2003), calves (average age: 4.2 d, n=19) were assigned randomly to one of two treatment groups. Treatment 1 calves (n = 9) were fed a 20% crude protein (CP): 20% fat milk replacer (**MR**) at a rate of 1.4% body weight of dry matter/d for 8 wk and treatment 2 calves (n = 10) were fed a 30% CP: 20% fat MR at a rate of 2.5% body weight of dry matter/d. No calf starter was offered. Composition of peripheral blood mononuclear cell (**PBMC**) populations collected at 4, 18, 32, 46, and 60 d of age was characterized by flow cytometry. The general responsiveness of PBMC populations was quantified using a DNA-synthesis (blastogenesis) assay; whereas, specific aspects of T and B cell function, T and natural killer cell function, and monocyte function were estimated *in vitro* using IgM, IFN-γ, and NO assays, respectively. Cell cultures were non-stimulated or stimulated with PWM, a T-cell dependent, B-cell mitogen (Franklin et al., 1994). From 11- to 60-d of age, the mean daily weight-gain of

treatment 2 calves (1.20 kg/d) exceeded the gains of treatment 1 calves (0.55 kg/d) (Fig. 1) and at 60-d of age, treatment 2 calves weighed 53% more than treatment 1 calves. Numbers of blood leukocytes and the composition of PBMC populations were unaffected by the plane of nutrition (Fig. 2). The $\gamma\delta TCR^+$ cell was the predominant lymphocyte subset at 4-d of age, a characteristic of PBMC populations in young ruminants (Hein and MacKay, 1991). The decline in this subset with increasing age is typical of young ruminants, including milk replacer-fed dairy calves. The increase in the percentage of B-cells with time, most notably from 32- to 60-d of age, also reflects immune maturation (Nonnecke et al., 1999). Mitogen-induced blastogenesis and IgM secretion were unaffected by treatment suggesting that T-cell proliferation and T-cell dependent, B-cell function are not influenced by nutrition. Mitogen-stimulated PBMC from intensified-diet calves; however, produced less IFN-γ and more NO in the latter weeks of the study suggesting that nutrition affects aspects of PBMC function associated with CMI (Fig. 3). The impact of altered IFN-γ and NO production on the calf's susceptibility to infectious disease are unknown. IFN-γ, crucial for effective responses to infection by intracellular pathogens, induces the activation of monocytes/macrophages resulting in the release of NO and its derivatives. NO plays an essential role in host defense, inflammation and immunity, although excess production promotes vasodilation, edema, and cytotoxicity leading to tissue damage (Abramson et al., 2001). Early weaning may limit negative consequences (if any) of excess NO production resulting from intensified nutrition.

In the second trial (Foote et al., 2005a), effects of nutrition and age on proliferation and activation calf lymphocyte subsets were investigated. Activation, differentiation, trafficking, and migration of T-cells through sites of inflammation/infection are essential for an effective IR. The interleukin (**IL**)-2 receptor (**CD25**) is expressed on activated T-cells, B-cells, and monocytes. Formation of the IL-2 receptor allows T-cell proliferation and differentiation to be driven by IL-2. In cattle, mitogen- and antigen-activated T-cells exhibit increased CD25 expression (Waters et al., 2003b). CD25 expression on T-cell subsets has been used to monitor responses of cattle to *Mycoplasma bovis* (Vanden Bush and Rosenbusch, 2003), bovine respiratory syncytial virus (Sanbulte and Roth, 2002), *M. bovis* (Nonnecke et al., 2005, Waters et al., 2003a) and mitogenic stimulation (Franklin et al., 1994). Leukocyte trafficking is regulated by surface adhesive interactions between T-cells or the extra-cellular matrix. Expression of the leukocyte adhesion molecule, **CD44,** is essential for movement of T-cells from the circulation into sites of inflammation and is up-regulated on antigen-activated T-cells and remains elevated on memory T-cells (7). In *M. bovis* infected cattle, CD44 expression is greater on antigen responsive T-cells than on unresponsive T-

cells (Waters et al., 2003b). L-selectin (**CD62L**), a lymph node homing receptor, is required for entry of cells into lymph nodes through high endothelial venules. Its expression on lymphocytes is down-regulated after polyclonal- and antigenic-induced activation (Dailey, 1998). Activated T-cells with reduced CD62L expression do not adhere to lymph node high endothelial venules in vitro or traffic to lymph nodes in vivo. In *M. bovis* infected cattle, decreased CD62L expression on antigen stimulated T-cells likely allows them to traffic to sites of infection (Waters, et al., 2003b).

Calves were fed a standard (20% CP, 20% fat, 0.45 kg/d) MR or an intensified (28% CP, 20% fat, 1.14 kg/d) MR from 1- to 8-wk of age. Blood was collected from calves (at 1-wk and at 8-wk of age) and from steers at the same times. Average daily weight-gain of intensified-diet (0.66 kg/d) calves was greater than standard-diet (0.27 kg/d) calves. Relative to mitogen-induced responses of T-cells from steers (5 to 6 m of age), T-cells from 1-wk old calves showed decreased proliferative activity, delayed increase in CD25 expression and no demonstrable changes in CD44 or CD62L expression. At wk 8 of age, the proliferation and expression of activation antigens by activated T-cells from standard-fed calves were comparable to responses of adult T-cells indicating rapid maturation of T-cell function during the neonatal period. Feeding an intensified MR was associated with decreased proliferation of mitogen-stimulated $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ cells (Fig. 4); decreased CD25 expression by mitogen-stimulated $CD4^+$ and $CD8^+$ cells; and decreased CD44 expression by mitogen-stimulated $CD8^+$ cells (Fig. 5). These data suggest that intensified nutrition influences CD25 expression by T-cell subsets from preweaned calves. Differences in the proliferation and CD44 expression may be due to the effects of intensified nutrition on CD25 expression. Reduced CD25 expression on T-cells resulting from increased dietary energy has been described in rodents (Peck et al., 2000). Intensified-diet calves also had lower CD62L expression on $CD8^+$ and $\gamma\delta^+$ TCR cells in non-stimulated cultures compared with standard-diet calves, suggesting that these subsets may not be cleared efficiently from the circulation as the same T cell subsets from standard-diet calves. Overall, these results indicate that the functional capacity of the calf's T-cell population becomes more adult-like during the first weeks of life and suggest that intensified nutrition influences T-cell activation during a period of rapid maturation of the neonatal immune system.

In a third trial (Foote et al., 2005b), effects of intensified nutrition on antigen specific, CMI responses of preweaned calves were examined. Calves were fed a standard (20% CP, 20% fat, 0.45 kg/d) MR or intensified (28% CP, 20% fat MR, 1.14 kg/d) MR from 1 to 6 wk of age. All calves were vaccinated with BCG at 1 wk of age as described (Nonnecke et al., 2005). The growth rate

of intensified-diet calves (0.62 kg/d) exceeded gain of standard-diet calves (0.29 kg/d). Liver, kidney, heart, thymus, and subcervical lymph nodes from intensified-diet calves were heavier than those from standard-diet calves (Table 1). Flow cytometric analysis of PBMC populations indicated that $CD4^+$ cell, $\gamma\delta TCR^+$ cell, and monocyte percentages, although unaffected by diet during the first 5 wk of the study, were higher in intensified-diet calves at wk 6. Percentages of $CD8^+$ T-cell or B-cell were not affected by diet. In intensified-diet calves, percentages of $CD4^+$ T-cells expressing IL-2 receptor increased and percentages of $\gamma\delta TCR^+$ T-cells expressing IL-2 r decreased with time. The same populations in standard-diet calves did not change with time. Percentages of $CD4^+$ and $CD8^+$ T-cells, and B-cells expressing major histocompatibility complex class II antigen were unaffected by diet or age. Although mitogen-induced IFN-γ and NO secretion increased with age for all calves, cells from intensified-diet calves produced less IFN-γ and more NO than those from standard-diet calves at wk 6 of the study (Figs. 6 and 7). These data are similar to those from the first trial (Fig. 3). In contrast, antigen (i.e. PPD)-induced IFN-γ and NO secretion were unaffected by diet (Figs. 6 and 7). Antigen-elicited delayed-type hypersensitivity, a measure of in vivo reactivity of T-cells, also was unaffected by diet suggesting intensified nutrition does not affect antigen-specific T-cell immunity in vivo (Fig. 8). Overall, these results suggest that intensified nutrition has minimal effects on the composition and functional capacities of PBMC populations from vaccinated calves.

In a recent review of the effects of early growth on the health and performance of heifers, Drackley (2005) suggested that nutritional sufficiency might be problematic for immune function during cold stress because maintenance requirements for thermo-regulation are increased. In a recent unpublished study, we evaluated the IR and health of calves fed traditional and intensive feeding programs under cold stress and thermo-neutral conditions. Sixty calves, 3 to 10 d old and weighing 40 to 45 kg, were assigned to a "cold environment" (room temperature 1 to 2°C with added humidity, n=30) or "thermo-neutral environment" (room temperature 15 to 16°C with no added humidity, n=30). Within each environment, calves were fed a traditional MR [20% CP:20% fat non-medicated, 0.45 kg/d with calf starter (18% non-medicated)] or an intensified MR [28% CP:20% fat MR, 1.14 kg/d with calf starter (22% non-medicated)]. All calves were vaccinated subcutaneously with OVA in Freund's incomplete adjuvant at the beginning of the trial (i.e. approximately 1-wk of age) and at 7-wk of age [results from an earlier trial demonstrating antibody responses of OVA sensitized calves are shown in Fig. 9 (left panel)]. Antigen-specific IgG_1 and IgG_2 concentrations in serum samples collected at 6, 7, and 8 wk of age were measured by ELISA [Fig.9, right panel

(IgG_1 only)]. Antibody responses of standard- and intensified-diet calves were comparable in the cold environment suggesting there may no benefit of intensified nutrition on adaptive IR of vaccinated, cold stressed calves. Secondly, antibody responses cold stressed calves were as vigorous as those of calves housed under thermo-neutral conditions. Additional research should evaluate effects of cold stress on broader aspects of immune function to confirm these preliminary observations.

Implications

We have evaluated the effects of intensified nutrition on the adaptive IR of the preweaned calves. To date, controlled studies have not demonstrated that increasing dietary protein and energy above traditional feeding programs benefits the adaptive IR of the preweaned calf. As consequence, antigen specific responses to vaccination and infection may not be enhanced by intensified nutrition. Ultimately, trials incorporating an infection model or surveying the incidence of natural infection in a large number of calves are necessary to establish effects of neonatal nutrition on calf health. Although not addressed, the systemic acute phase response characteristic of many calf-hood infections may place metabolic demands on the calf that can only be met by intensified nutrition programs.

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Figure 1. Growth performance (Nonnecke et al., 2003)

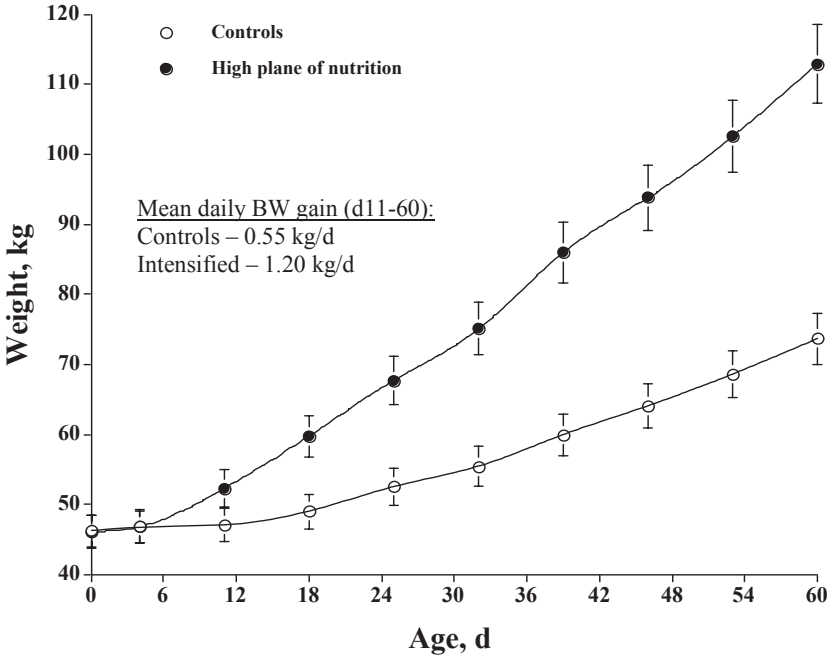

Figure 2. T-cell percentages in peripheral blood (Nonnecke et al, 2003)

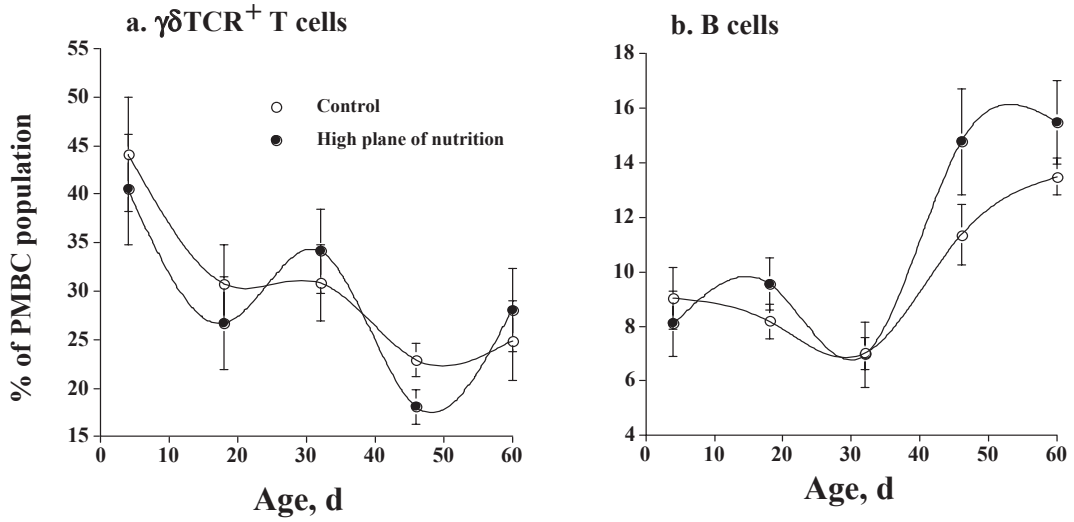

Figure 3. Interferon-γ and nitric oxide production (Nonnecke et al, 2003)

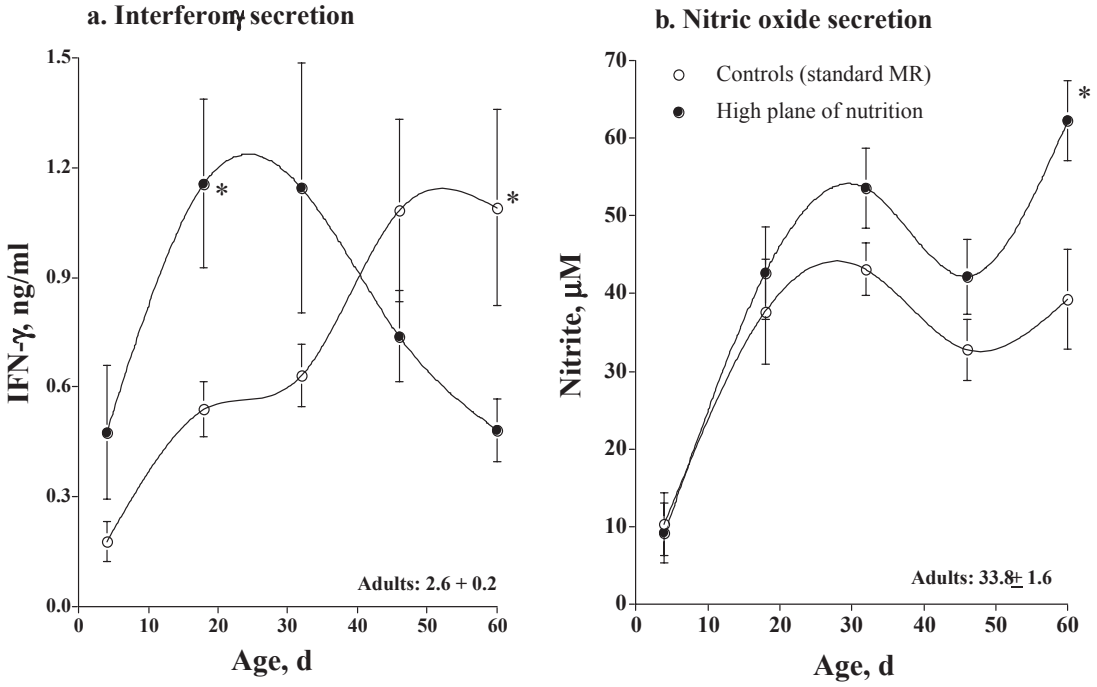

Figure 4. T-cell subset proliferation (Foote et al., 2005b)

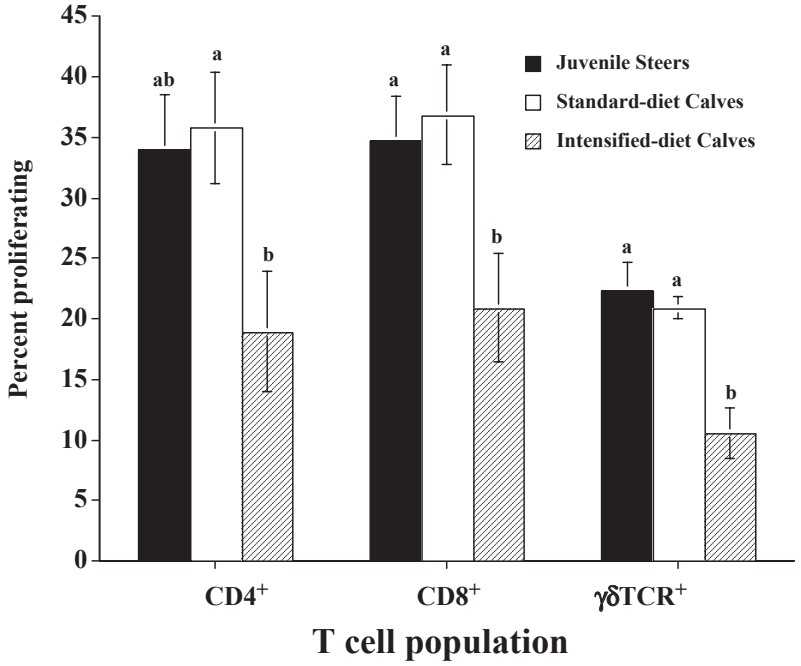

Figure 5. T-cell subset activation (Foote et al., 2005b)

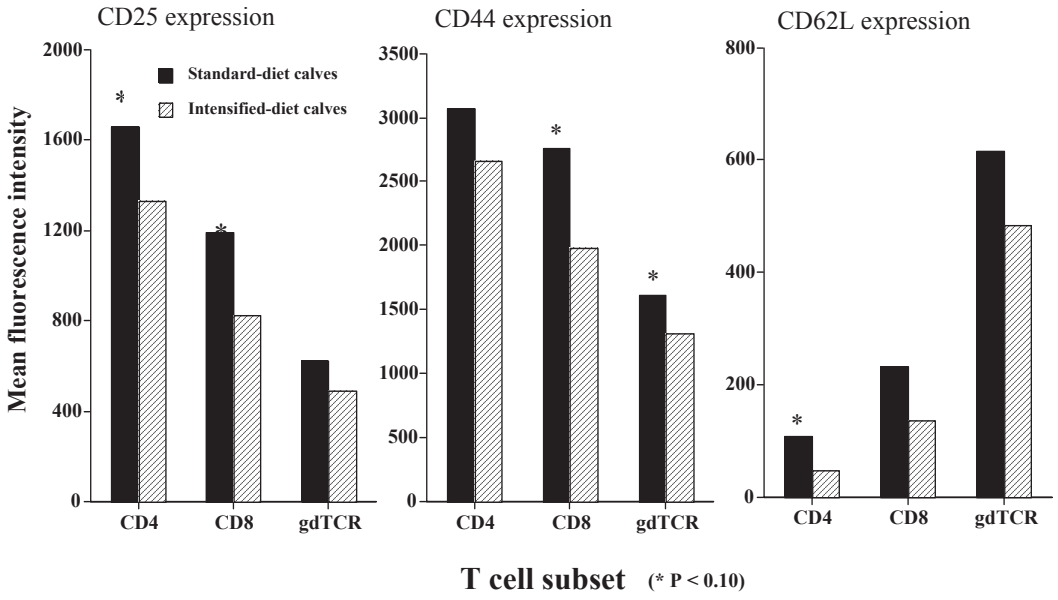

Figure 6. Polyclonal- and antigen-induced interferon-γ responses compared (Foote et al., 2005a)

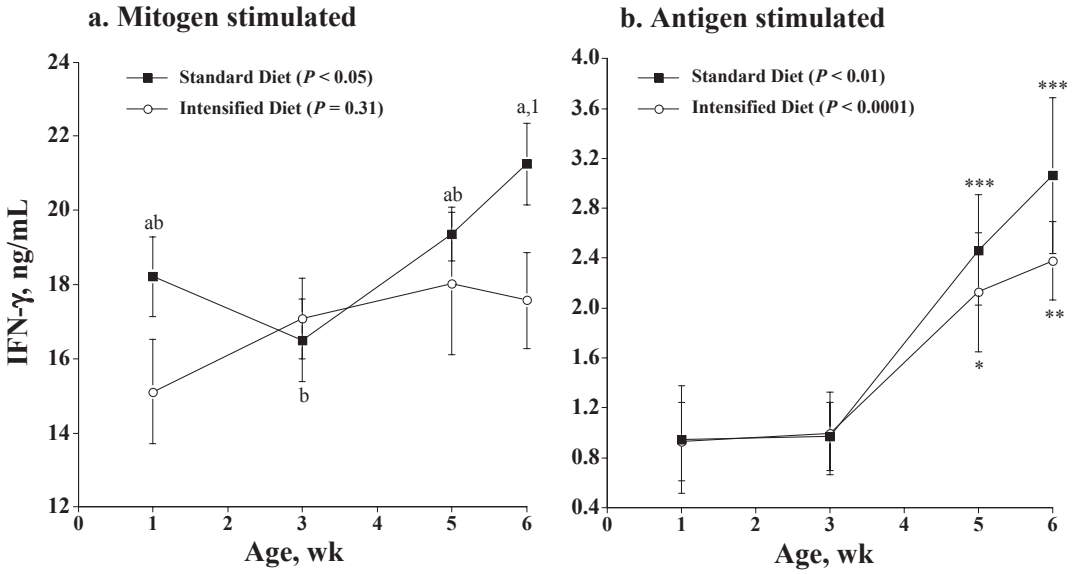

Figure 7. Polyclonal- and antigen-induced nitric oxide responses compared (Foote et al., 2005a)

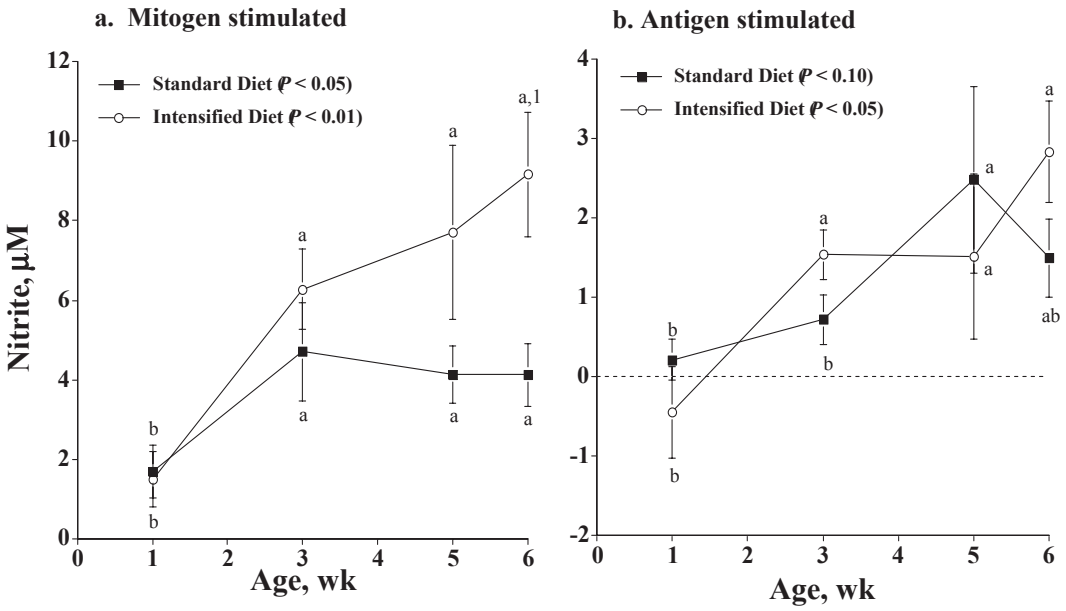

Figure 8. In vivo responsiveness to recall antigen (Foote et al., 2005a)

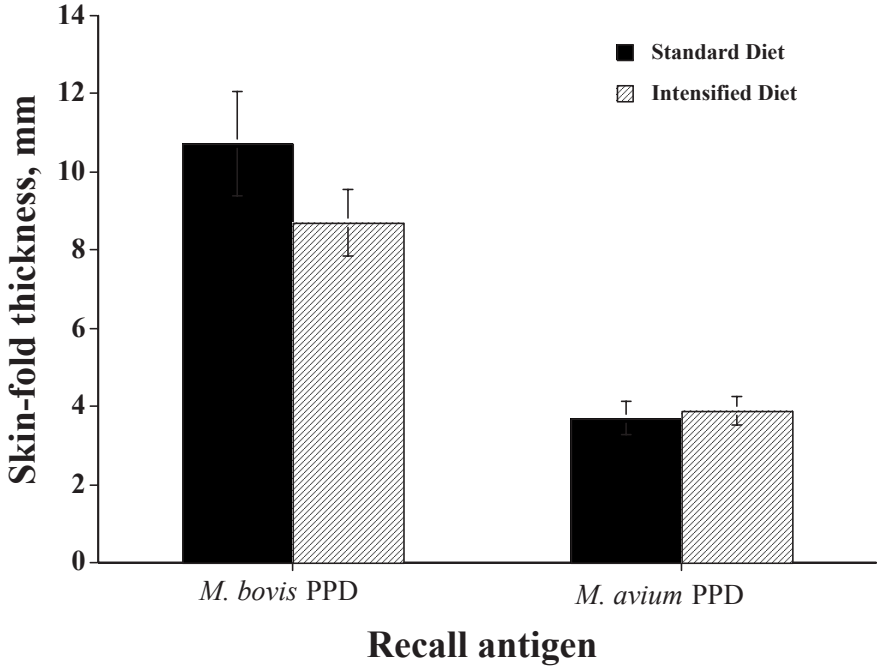

Figure 9. Antibody (IgG1) production by calves sensitized to ovalbumin (OVA): effects of neonatal nutrition.

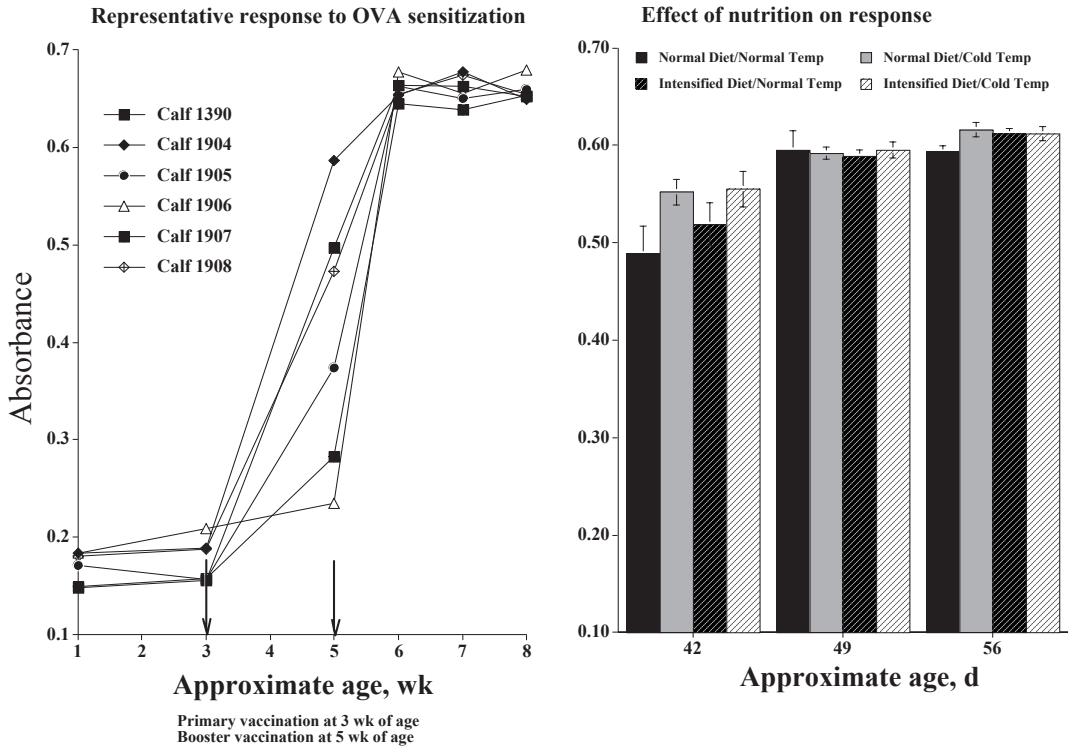

Table 1. Weights of organs (means ± SE) of immunological and metabolic importance from 6-wk-old calves fed standard (n=11) and intensified diets (n=11) from 1 to 6 wk of age (Foote, e al., 2005b).

Organ	Standard Diet	Intensified Diet	*P* value
Subcervical Lymph Node, g	7.75 ± 0.55	9.90 ± 0.70	< 0.05
Prefemoral Lymph Node, g	4.82 ± 0.88	6.75 ± 1.06	NS[1]
Thymus, g	178.39 ± 22.58	337.68 ± 37.88	< 0.01
Liver, kg	1.12 ± 0.04	1.67 ± 0.08	< 0.0001
Adrenal Gland, g	4.49 ± 0.24	5.92 ± 0.26	< 0.001
Pancreas, g	48.95 ± 8.85	45.64 ± 3.30	NS
Thyroid Gland, g	12.81 ± 1.49	16.12 ± 2.16	NS
Kidney, g	309.27 ± 11.95	433.43 ± 27.37	< 0.001
Heart, g	420.60 ± 16.29	544.81 ± 36.73	< 0.01

[1]NS = Not significant ($P > 0.1$).