Ontogeny of the Immune Response: Effect of Protein Energy Malnutrition in Neonatal Calves

Philip J. Griebel, Matt Schoonderwoerd and Lorne A. Babiuk*

ABSTRACT

Immunocompetence of neonatal, Holstein bull calves fed for maximal growth (Control; n=4) or protein energy malnutrition feeding (PEM; n=4) for four weeks was assayed *in vitro* and *in vivo*. All calves exhibited elevated cortisol levels for ten days postnatally. At this time calves also were neutrophilic and lymphopenic. In addition lymphocyte function, as measured by lymphocyte proliferation and interleukin-2 activity, was reduced at this time as compared to older calves. After two weeks of protein energy malnutrition feeding, calves had significantly lower body weight, lymphocyte interleukin-2 activity and lymphocyte proliferation when compared with age-matched controls. Two weeks after protein energy malnutrition ration reversal, interleukin-2 activity and lymphocyte proliferation was comparable for both groups. There was no significant difference in serum cortisol concentration between control and protein energy malnutrition calves. The kinetics of the protein energy malnutrition group's primary humoral immune response was retarded, thus significantly lower antibody levels to K99 antigen were observed 8 to 12 days postimmunization. There was no significant difference between groups when comparing secondary response to K99 antigen.

Key words: Neonatal calf, malnutrition, cortisol, interleukin-2, cellular immunity, humoral immunity.

RÉSUMÉ

Cette expérience consistait à vérifier, *in vitro* et *in vivo*, durant quatre semaines, l'immunocompétence de huit veaux Holstein, mâles et nouveau-nés, dont quatre recevaient une ration destinée à favoriser une croissance maximale, tandis qu'on donnait aux quatre autres une ration déficiente en protéines, ainsi qu'en énergie, et susceptible d'engendrer un état de malnutrition. Durant les dix jours ultérieurs à leur naissance, tous les veaux affichèrent un taux sérique élevé de cortisol, ainsi qu'une neutrophilie et une lymphopénie. De plus, la fonction des lymphocytes, telle que mesurée par la prolifération des lymphocytes et l'activité de l'interleucine #2, subit une baisse, durant cette période, par rapport à un âge plus avancé. Au bout de deux semaines, les veaux qui recevaient la ration déficiente affichèrent une diminution significative du poids corporel, de l'activité de l'interleucine #2 et de la prolifération des lymphocytes, par rapport à des veaux témoins du même âge. Deux semaines après la correction de la ration déficiente, l'activité de l'interleucine #2 et la prolifération des lymphocytes se révélèrent comparables chez les veaux des deux groupes expérimentaux et on n'enregistra pas de différence significative dans la teneur de leur sérum en cortisol. La cinétique de la réponse immunitaire humorale primaire afficha du retard chez les veaux qui recevaient la ration déficiente, de sorte qu'on enregistra des taux significativement plus faibles d'anticorps contre l'antigène K99, de huit à 12 jours après la vaccination. Une comparaison de la réponse secondaire à l'endroit de cet antigène ne révéla toutefois pas de différence appréciable entre les veaux des deux groupes expérimentaux.

Mots clés: veau nouveau-né, malnutrition, cortisol, interleucine #2, immunité cellulaire, immunité humorale.

INTRODUCTION

About 75% of the mortality in dairy animals, less than one year of age, occurs during the neonatal period (less than 28 days of age) (1). A major factor contributing to this high mortality is the feeding of either a suboptimal quantity or quality of milk replacer. During a recent field investigation, on 20 dairy farms, it was evident that the use of inferior milk replacers was a common practice. These products contained plant derived protein and/or overheated milk proteins, which the preruminant calf cannot digest adequately (2). The use of these products results in marked weight loss, nutritional scours, increased susceptibility to infectious diarrhea and pneumonia, and a high mortality (2). In children there is an increased incidence and severity of infectious disease, secondary to protein energy malnutrition (PEM) induced impairment of the immune system (3,4,5,6). It is possible that a similar effect occurs in neonatal calves and contributes to the increased mortality.

Information about the influence of PEM on bovine immunity has been limited to mature cattle (7). Prolonged PEM resulted in thymic atrophy with no lymphoid depletion of lymph nodes and spleen (7). In addition there was no significant depression of humoral or cell-mediated immunity (7). These data may not be applicable to neonatal calves, which have an immature immune system (8). In the neonatal calf there are age-related increases in peripheral blood T-cell populations (10), neutrophil functions (11), complement activation (12,13), delayed hypersensitivity (14,15) and lymphocyte proliferation responses (9,16). The decreased immune function in the

*Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0 (Griebel, Babiuk) and Alberta Agriculture Pathology Laboratory, Edmonton, Alberta (Schoonderwoerd).

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calf was most pronounced during the first two weeks of life (9).

Two recent studies provide indirect evidence that PEM may decrease the immunocompetence of neonatal calves. In one study marked thymic atrophy was observed in neonatal calves receiving electrolytes solely for seven days (17). In the second study histological examination of 20 emaciated neonatal calves revealed marked lymphoid depletion in thymus, spleen, lymph nodes and aggregated lymphoid follicles consistent with cellular immunodeficiency (Unpublished data). Precocious thymic involution in malnourished children has been related to decreased immunocompetence (4,18).

Nutritional deficiency has diverse immunological effects including decreased cell-mediated immunity, T-cell numbers, helper T-cell function, complement activity and neutrophil bacterial killing capacity (13,18,19,20). Many of these functions are already suboptimal in neonatal calves (8). This may make adequate nutrition of critical importance in the prevention of infectious disease during this time. The purpose of this study was to investigate the effects of PEM on the ontogeny of immunocompetence in neonatal Holstein calves.

MATERIALS AND METHODS

CALVES AND EXPERIMENTAL DESIGN

Eight healthy, newborn Holstein bull calves were obtained from four dairy farms in the Saskatoon area. All calves were less than two days old and had received 4 L of colostrum in the first 24 h of life.

On arrival at the Western College of Veterinary Medicine calves were assigned alternately to the control or PEM group. Calves were individually housed in an isolation room. The guidelines of the Canadian Council on Animal Care, "Guide to the Care and Use of Experimental Animals", Volume 1, were followed. Room temperature was maintained at 14-16°C.

Calves were fed twice daily with high conversion milk replacer (Grober Limited, Cambridge, Ontario). This product contains 20% protein (all milk derived), and 15% fat on a dry matter basis. Once dissolved in warm water, the solution consisted of 12.5% dry matter. Control calves were fed for maximum growth on the feeding schedule shown in Table I.

TABLE I. Feeding Schedule for Control Calves

Day	Amount of Powder Twice Daily (g)
1-4	225
5-7	250
8-10	275
11-13	300
14-16	315
17,18	330
19,20	350
21,22	390
23,24	450
25,26	500
27,28	560
29-32	630
33-36	700
37-42	750

Protein energy malnutrition calves received 50% of maintenance requirements until day 28 (110-130 g of powder twice daily). The maintenance requirements for metabolizable energy in the neonatal calves was calculated using body weight and 450 kJ per $kg^{.75}$. One kg of high conversion milk replacer is equivalent to 16.5 mJ metabolizable energy. On day 29, until the end of the experiment, the PEM calves' dry matter intake for milk replacer was increased to that of the control group. Starter pellets (18% protein), mixed equally with rolled oats and alfalfa pellets, were provided *ad libitum* after day 42 while milk replacer dry matter remained constant. Calves were weighed weekly, before the morning feeding, and body temperature was monitored daily.

CORTICOSTEROID LEVEL

Concentrations of serum cortisol were measured by competitive radioimmunoassay with the Amerlex cortisol kit (Amersham, Oakville, Ontario). Serum samples were collected weekly, and held at -20°C until assayed.

PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) CULTURE AND BLASTOGENIC ASSAYS

Peripheral blood was collected into citrated dextrose. Peripheral blood mononuclear cells were isolated on percoll (Pharmacia, Dorval, Quebec) gradients with a density of 1.078 g/mL, washed three times with Hanks' balance salt solution (HBSS; Gibco Canada Ltd., Burlington, Ontario) and then suspended in RPMI-1640 (Gibco Canada Ltd., Burlington, Ontario).

The blastogenic responses of PBMC were measured by incubating quadruplicate cultures of 1×10^5 cells, with a total medium volume of 0.2 mL in 96-well microtiter plates (Linbro; Flow Laboratories, Mississauga, Ontario). All cultures were duplicated using 5% fetal bovine serum (FBS) or 5% autologous heat-treated serum. Blastogenic responses were consistently greater with autologous sera and only these data are reported.

Peripheral blood mononuclear cells were cultured with and without concanavalin A (Con A; 5 μg/mL; Calbiochem-Behring, La Jolla, California) or purified human recombinant interleukin-2 (rIL-2: kindly provided by Dr. P.E. Baker, Immunex Corporation, Seattle, Washington). The rIL-2 was used at a final concentration of 20 units/mL, previously found to be optimal. Cultures were incubated at 37°C for 72 h in a humidified 5% CO_2 atmosphere. During the last 18 h incubation 1.0 μCi methyl [^{3}H] thymidine ([^{3}H]TdR; Amersham, Oakville, Ontario) was added to each culture in 20 μL. Cells were harvested with a MASH harvestor (Flow Laboratories, Mississauga, Ontario). Incorporation of [^{3}H]TdR was quantitated using a Beckman LS 8000 liquid scintillation counter (Beckman Instruments Inc., Mississauga, Ontario). The incorporation of [^{3}H]TdR is expressed as mean counts per minute (cpm) per culture. The value of one standard deviation was consistently less than 10% for each animal. Peripheral blood mononuclear cells from a four month old Holstein bull calf was included in all blastogenic assays as control for interassay variability.

INTERLEUKIN-2 GENERATION AND QUANTITATION

For each animal, triplicate microtiter cultures of 3×10^6 PBMC were plated in 0.3 mL RPMI-1640 with 5% FBS and Con A at a final concentration of 5 μg/mL. After incubation at 37°C for 24 h, in a humidified 5% CO_2 atmosphere, supernatant was collected. Cells were removed, after centrifugation at 600 × *g* for 5 min at 4°C, and the supernatant was stored at 4°C until tested within two weeks.

Interleukin-2 (IL-2) activity was quantitated at Immunex Corporation (Seattle, Washington) using a bovine IL-2 dependent lymphoblastoid cell line (21). Interleukin-2 activity (units/mL) was determined by a computer-assisted logit analysis of the data (22). The mean of triplicate cultures was used in all statistical analyses. For each animal, the value of one standard deviation was consistently less than 15%. Peripheral blood mononuclear cells from a four month Holstein bull calf were included in all assays as control for interassay variability.

HEMATOLOGY

Blood samples were collected in heparinized vacuum tubes (Becton Dickinson, Rutherford, New Jersey) and total and differential leukocyte counts, complete blood count (CBC), and fibrinogen levels were determined. Samples were processed by the Department of Clinical Pathology, Western College of Veterinary Medicine, using routine methodology.

IMMUNIZATION WITH K99 ANTIGEN

Purified K99 pili antigen (VIDO, Saskatoon, Saskatchewan) was suspended in aluminum hydroxide. Primary immunization of all calves was done on day 23, five days before the PEM group returned to control diet. K99 antigen (500 μg in 0.5 mL) was injected intramuscularly. Serum samples were collected prior to and following immunization for 20 days, at four day intervals. Serum samples were held at -20°C until assayed.

Secondary immunization took place 60 days later (day 83) using the same dose and route of administration. Serum samples were collected prior to and following secondary immunization for two weeks, every five days.

A passive hemagglutination (HA) test (23) was used to determine the K99 antibody titer. Formaldehyde-fixed, tannic acid treated goose red blood cells were sensitized with purified K99 pili antigen. All serum samples were analyzed within the same assay.

DATA ANALYSIS

Individual time point comparisons for group hematology values, serum cortisol concentrations, and body weight were made using the Student's t test. A one-way analysis of variance (ANOVA) was used to analyze changes within groups over time. The Student-Newman-Keuls procedure was used to detect differences among means when ANOVA revealed a significant ($P<0.05$) change.

Control and PEM IL-2 activity, lymphocyte proliferation responses and K99 HA titer were compared at each time point using the Mann-Whitney test. Friedman's analysis of variance by ranks was used to analyze changes within groups for IL-2 activity and lymphocyte proliferation. A Multiple Comparison Procedure for use with Friedman's ANOVA was used to detect differences when Friedman's ANOVA revealed a significant ($P<0.05$) change (24).

RESULTS

BODY WEIGHT

There was no significant difference in initial body weight for both groups. During the first four weeks, control calves gained weekly approximately 1 kg, while PEM calves lost 1 kg per week (Fig. 1). After two weeks, PEM calves had a significantly lower body weight than age-matched controls. The PEM calves, at four weeks of 50% maintenance, remained bright with no loss of appetite. These calves were noticeably weaker and preferred to remain recumbent when being handled. Body temperature of all calves was within the normal range throughout the experiment.

The PEM calves started to gain weight within three days after returning to the control diet. There was no diarrhea associated with the abrupt increase of dry matter. Two weeks after the reversal of PEM, the significant difference in body weight between groups was still present.

SERUM CORTISOL

There was no significant difference in serum cortisol profiles between groups (Fig. 2). Both had a significant cortisolemia on day 3, which declined

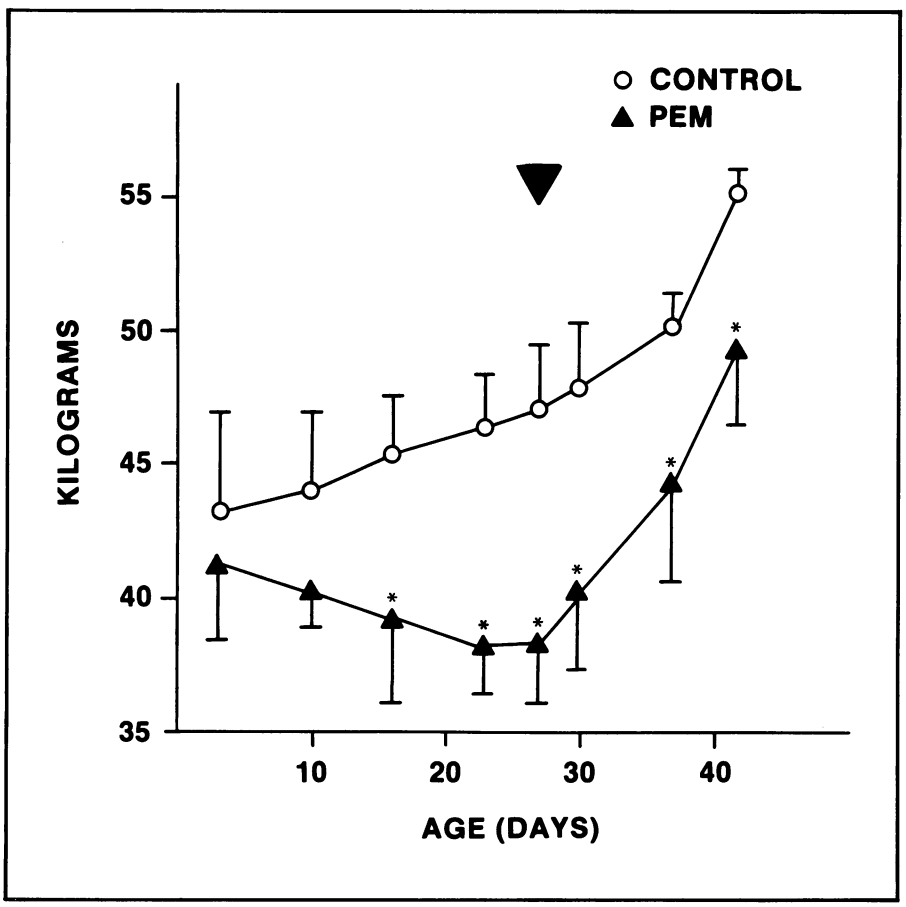

Fig. 1. Body weight of neonatal calves. Values are mean and SD. PEM group weight different from controls at points indicated by *($P<0.01$). Arrowhead indicates the day when PEM calves returned to full maintenance ration.

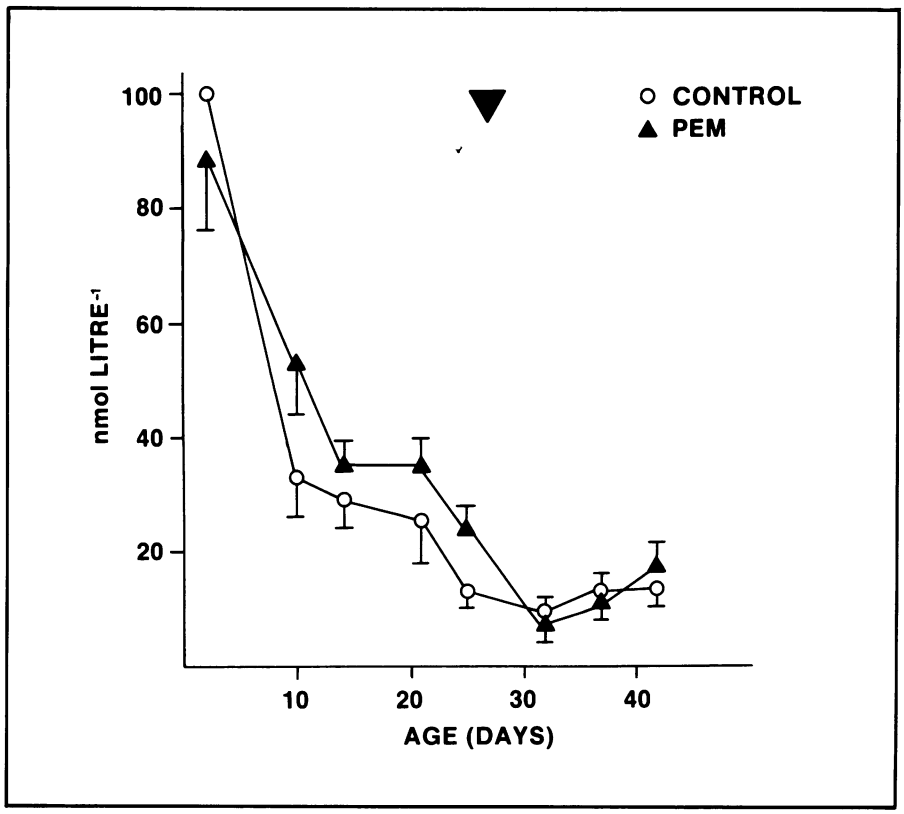

Fig. 2. Serum cortisol levels in neonatal calves. Values are mean and SD for each group. Arrowhead indicates the day when PEM calves were returned to full maintenance ration.

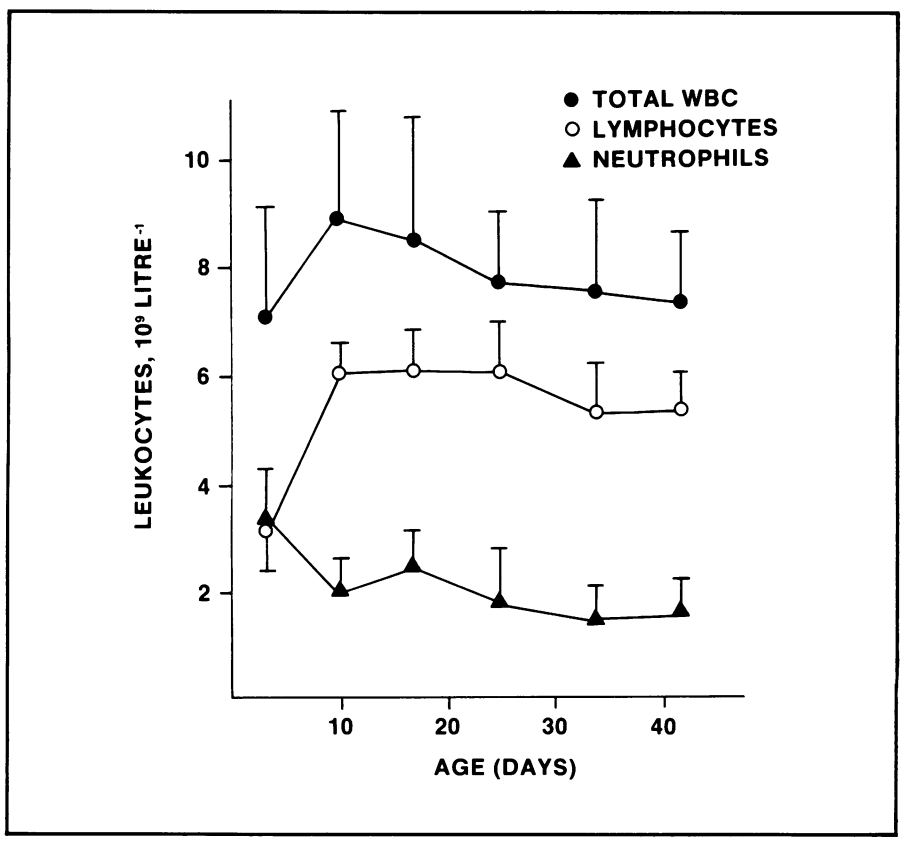

Fig. 3. Leukocyte numbers of combined control and PEM groups. These groups did not differ significantly at any age assayed. Data represents mean and SD. Total WBC (O) did not differ significantly at any age. Lymphocyte (O) and neutrophil (△) counts were different at three days at $P<0.01$.

during the next ten days. Baseline levels of serum cortisol were present after three weeks of age.

HEMATOLOGY

At no time during the experiment did the control and PEM group differ significantly in hematological parameters measured. Hemoglobin, PCV, RBC, total protein and fibrinogen concentration did not change significantly with age. Fibrinogen levels remained below 5.3 g/L for all calves.

The only significant hematological changes with age were neutrophilia and lymphopenia observed on day 3 (Fig. 3).

IL-2 ACTIVITY

On day 3 there was no significant difference in the IL-2 activity between the two groups (Fig. 4). Controls had a significantly increased IL-2 activity in all assays subsequent to day 3. The IL-2 activity of the control group was not significantly different, following day 10, when compared with the four month old Holstein bull calf.

Not until day 42, two weeks after PEM reversal, did the PEM group have a significant increase in IL-2 actvity when compared to the day 3 assay. The IL-2 activity in the PEM calves was significantly below the four month old calf until one week after PEM reversal.

When comparing both groups there was a significant depression of IL-2 activity in the PEM group between day 10 to 28. This difference was absent one week after PEM diet reversal due principally to the rising IL-2 activity in the PEM calves.

LYMPHOCYTE PROLIFERATION RESPONSES

A similar ^{3}H-TdR incorporation profile was observed for the control group in the three *in vitro* assays (Fig. 5). Lymphocyte proliferation (LP) increased significantly after day 10 for PBMC stimulated with rIL-2 and on day 10 with Con A. At six weeks LP in the control calves had declined in all assays to a level comparable with day 3 assays and the four month old calf.

During the first ten days PBMC cultured from PEM calves, with and without mitogen, responded with similar proliferation values as PBMC from control calves. Between the second and fourth week the LP of PEM calves

was significantly lower when compared to age-matched controls. When compared to day 3 assays, the PBMC response, in the PEM group, to rIL-2 and Con A increased significantly between the second and fourth week. Malnourishing calves, during this period, did not eliminate the increase in LP but did decrease its amplitude. The exception was the PEM group PBMC cultured without mitogen. Lymphocyte proliferation in this assay declined throughout the experiment when compared to day 3.

The difference in LP responses between the two groups persisted one week after PEM reversal. At this time there was no significant difference in rIL-2 induced LP but Con A induced LP of the PEM group increased significantly compared to the controls.

K99 HUMORAL RESPONSE

All calves in the control group had peak titers of K99 antibody 12 days after primary immunization (Fig. 6). Protein energy malnutrition calves were slower in their response, requiring 20 days for all calves to attain an equivalent peak titer. This lag made the PEM group titers significantly lower than the control group, between days 8 and 12 postimmunization. The peak titers of PEM calves, after primary immunization, was not significantly different from the controls. The K99 antibody titers of both groups remained stable during the 60 days following primary immunization. There was no significant difference between groups in secondary humoral response amplitude or kinetics. Both groups had peak titers by day 10 postimmunization.

DISCUSSION

Body weight could provide a diagnostic aid when investigating a calf disease problem where malnutrition is a differential diagnosis. The birth weight of the eight male calves in this experiment was 43.9 ± 3.8 kg, which was consistent with a previous report (17). Based on this experiment, neonatal Holstein bull calves would be expected to be 1 kg above birth weight for each week of age when fed for maximum growth and housed at thermoneutrality. Calves should have significantly lower body weights after two weeks of a PEM ration (Fig. 1). Correction of the dietary deficit, if this is the primary problem, should be followed by rapid weight gain.

The PEM diet in this experiment did not significantly alter any hematological parameters measured. Prolonged PEM in humans has been associated with hypohemaglobinemia, anemia and lymphopenia (13). However, age of host, type and duration of malnutrition and possible concurrent infection may all significantly alter hematological values (25). Under the experimental conditions of this study the hematology parameters used did not provide a diagnostic aid in cases of PEM in neonatal calves. Hematology may be valuable in differentiating between PEM and bovine viral diarrhea (BVD) infection of neonatal calves. Both diseases may result in lymphoid depletion of primary and secondary lymphoid organs but with BVD a severe lymphopenia has also been observed (26).

The lymphopenia and neutrophilia observed on day 3 (Fig. 3) were consistent with previous reports on neonatal calf hematology and the presence of elevated serum cortisol (27,28). Suppression of LP and lymphocyte IL-2 activity at this time (Fig. 4) may also be the result of the elevated cortisol. Both *in vitro* and *in vivo* studies have established that cortisol can suppress these lymphocyte functions in the bovine (29), human, rat and mouse (30).

The PEM diet did not significantly alter serum cortisol concentration of neonatal Holstein bull calves (Fig. 2). Thus the suppression of lymphocyte function observed during PEM (Fig. 4) would not appear attributable to changes in serum cortisol. However, in young rats a very narrow range of serum corticosterone was identified as compatible with normal thymus mass (31) and changes in thymic mass have been correlated with altered immune function (18). Although serum cortisol did not differ significantly between groups there was a consistent elevation of mean cortisol levels in the PEM group. At this time cortisol should not be completely dismissed as a possible mediator of PEM induced suppression of lymphocyte function.

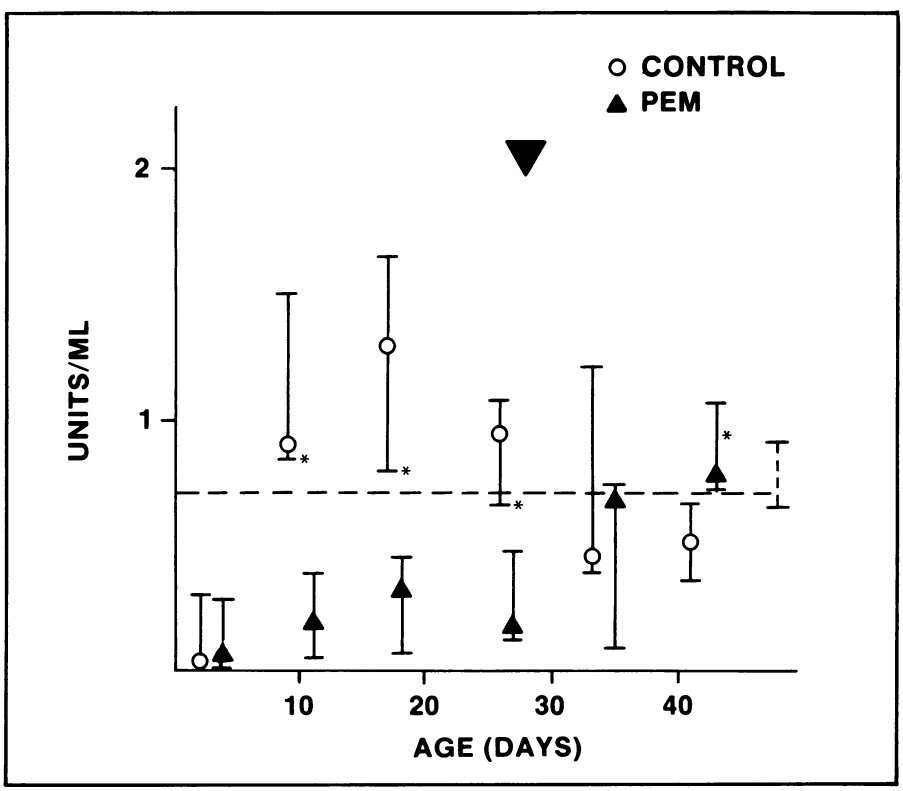

Fig. 4. Interleukin-2 activity of neonatal calves assayed at weekly intervals. Values expressed as median and range for each group. A four month old calf (---) was included as a control for interassay variation. Control group values are different from PEM group at points indicated by *($P<0.05$). Arrowhead indicates the day when PEM calves were returned to full maintenance ration.

As serum cortisol declined there was a significant rise in control group IL-2 activity (Fig. 4), IL-2 responsiveness and LP (Fig. 5). This elevated LP was observed with and without mitogen (background) using either FBS or autologous serum. Thus a soluble factor was not responsible for stimulating this rise in LP. Lymphocyte activation is associated with an increased ability to produce IL-2 and respond to this interleukin (32). The observed increase in these lymphocyte functions, in neonatal calves' PBM, would be consistent with the occurrence of an increasing population of activated T-cells. The occurrence of an elevated number of activated T-cells in neonatal calf PBM two to four weeks postnatally may be a normal event in immunocompetence ontogeny. A population of short-lived, rapidly dividing, cortical thymus-derived T-cells has been identified in neonatal mice two to three weeks postnatally (33,34). During this same period there was a rapid increase in the number of IL-2 producing T-helper cells (35).

Control group IL-2 activity (Fig. 4), IL-2 responsiveness and LP (Fig. 5) declined after four weeks to a level comparable with the four month old calf. These diminished T-cell responses were probably not secondary to altered monocyte/macrophage function which does not change significantly during this period (36). It is possible that the decline in function may result from a reduced number of T lymphocytes in the percoll isolated PBMC. The number of B-cells in the peripheral blood of neonatal calves increases significantly after 20 days and this may produce a relative decrease in the percentage of T-cells (36). Alternatively, the decline in the T-cell functions assayed may be the result of developing regulatory cell function which suppresses these responses.

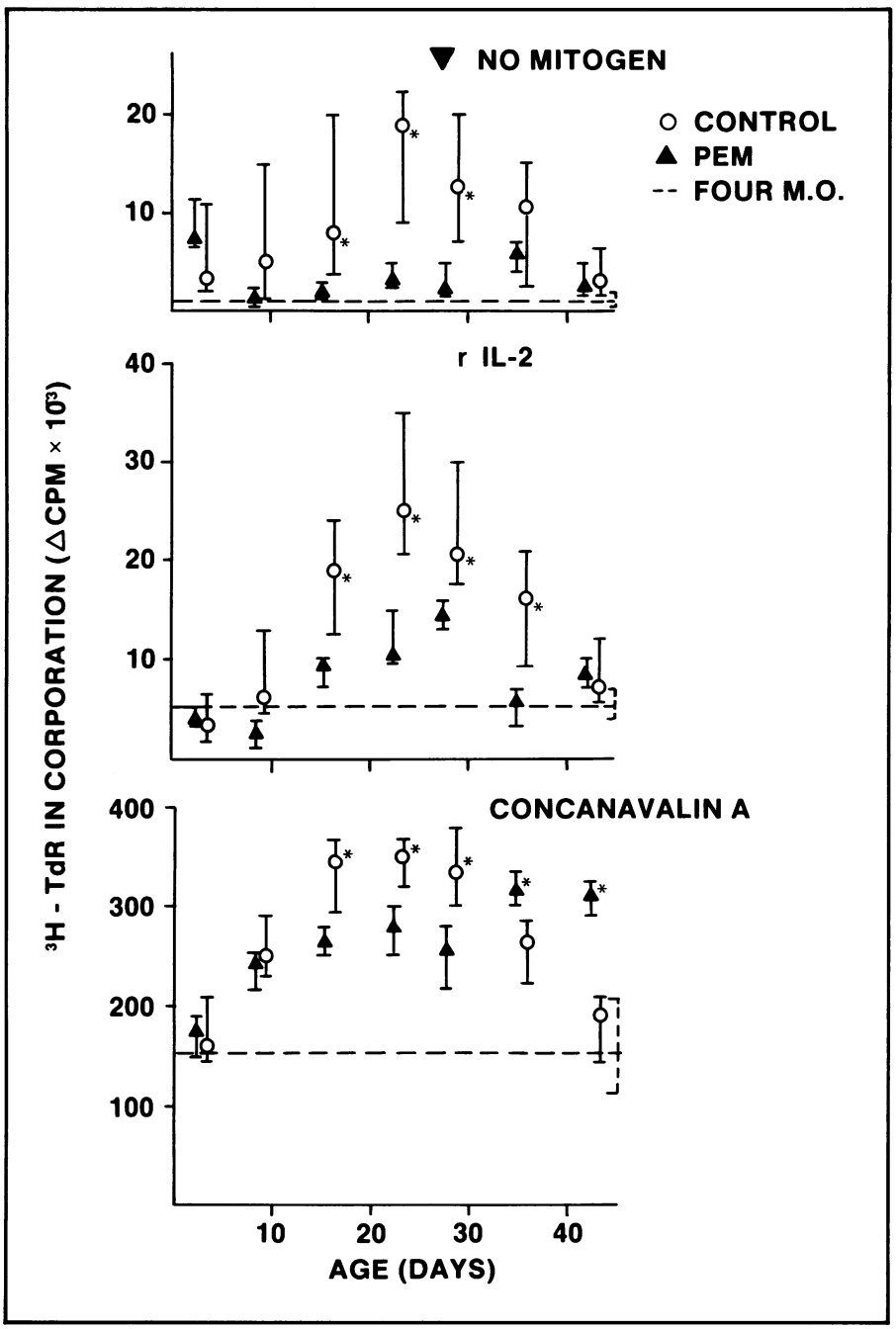

Fig. 5. Lymphocyte proliferation responses of neonatal calves. Values are expressed as median and range for each group. A four month old calf (---) was included as a control for interassay variation. Control values are different from PEM group at points indicated by *($P<0.05$). Arrowhead indicates the day when PEM calves were returned to full maintenance ration.

Protein energy malnutrition had a major effect on the development of IL-2 activity in neonatal calves (Fig. 4). Only after reversal of PEM did IL-2 activity rise significantly above that of day 3 to be comparable with the control group and the four month old calf. Loss of T-helper function has been one of the major T-cell defects consistently associated with PEM in humans (19,20). This results from both a numerical and functional deficiency of T-helper cells. These cells, through the production of IL-2, play an integral role in both humoral and cell-mediated immune responses (37). Interleukin-2 is an essential signal in the clonal expansion of effector cells in both these arms of the immune system. Thus the loss of T-helper function, evident by the decreased IL-2 activity, could be responsible for both the suppressed LP and delayed humoral response to K99 antigen observed during PEM. An equivalent peak K99 titer was delayed eight days for the PEM fed group (Fig. 6). This delay coincided with the recovery time of PBM IL-2 activity after termination of the PEM diet. If PEM feeding had been prolonged the primary antibody response of these calves may have been more severely inhibited and never been comparable to the controls.

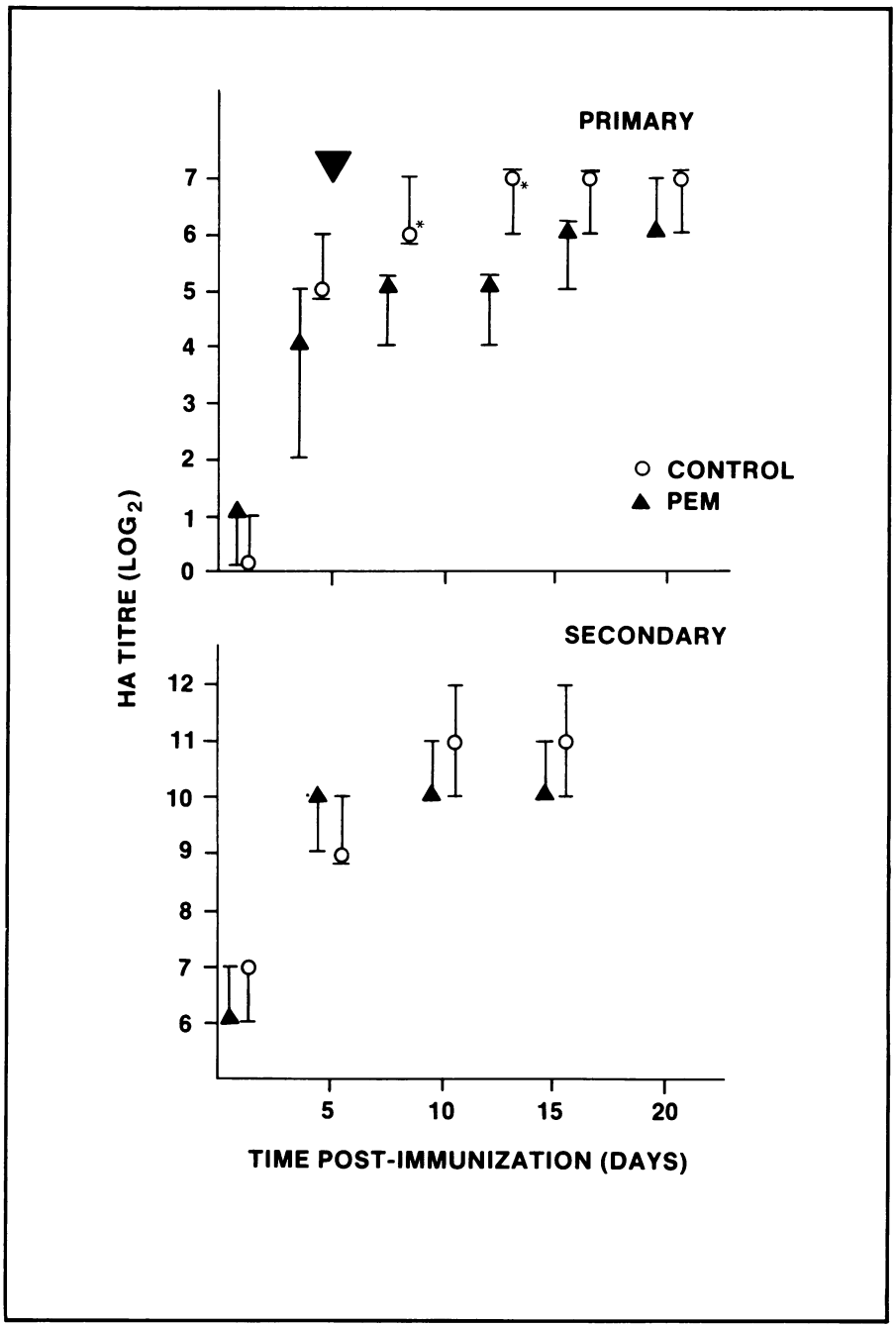

Fig. 6. Hemagglutination titer of neonatal calves following primary and secondary immunization with purified K99 pili antigen. Values are expressed as median and range for each group. Control group values are different from PEM group at points indicated by *($P<0.05$). Arrowhead indicates the day when PEM calves were returned to full maintenance ration.

In this study a major effect of PEM feeding was the reduction of immune response amplitude. The feeding of a poor quality milk replacer, which also limits available metabolizable energy, would be expected to have a similar effect. The effect of feeding an inadequate quantity or quality of milk replacer would be further exacerbated if the calves were housed in an environment below thermoneutrality which increases the energy requirement. Although neonatal calves may respond immunologically to a pathogen, PEM may limit this response, with a possible inability to completely clear the infection. The resulting chronic infections may be important in further debilitating these animals and contributing to increased mortality and lost productivity.

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REFERENCES

1. **RADOSTITS OM, BLOOD DC.** Herd health. Philadelphia: WB Saunders, 1985: 116-140.
2. **ROY JHB.** Diarrhea of nutritional origin. Proc Nutr Soc 1969; 28:160-168.
3. **CHANDRA RK, NEWBERNE PM.** Nutrition, immunity and infection. New York: Plenum Press, 1977: 67-126.
4. **EDELMAN R.** Cell mediated immune response in protein calorie malnutrition - a review. In: Suskind RM, ed. Malnutrition and the immune response. New York: Raven Press, 1977: 47-75.
5. **SCRIMSHAW NS, TAYLOR CE, GORDON JE.** Interactions of nutrition and infection. Geneva: World Health Organization, 1968: 60-142.
6. **CHANDRA RK.** Immunodeficiency in undernutrition and overnutrition. Nutr Rev 1981; 39:225-242.
7. **FRISKE RA, ADAMS LG.** Immune responsiveness and lymphoreticular morphology in cattle fed hypo- and hyperalimentation diets. Vet Immunol Immunopathol 1985; 8:225-244.
8. **OSBURN BI, STABENFELDT GH, ARDANS AA, TRESS C, SAWYER M.** Perinatal immunity in calves. J Am Vet Med Assoc 1974; 164:295-298.
9. **ROSSI CR, KIESEL GK, HUDSON RS.** Kinetics of detection of blastogenic responses of neonatal calves inoculated in utero with tetanus toxoid, killed *Mycobacterium bovis* and killed *Brucella abortus*. Am J Vet Res 1979; 40:576-579.
10. **OUTTERIDGE PM, DUFTY JH.** Surface markers for characterization of bovine blood lymphocyte populations and changes in these from birth to maturity. Res Vet Sci 1981; 31:315-322.
11. **HAUSER MA, KOOB ND, ROTH JA.** Variation of neutrophil function with age in calves. Am J Vet Res 1986; 47:152-153.
12. **RENSHAW HW, ECKBLAD WP, TASSINARI PD, EVERSON DO.** Levels of total hemolytic complement activity in paired dairy cow-newborn calf sera. Immunology 1978; 34:801-805.
13. **RENSHAW HW, EVERSON DO.** Classical and alternate complement pathway activities in paired dairy cow-newborn calf sera. Comp Immunol Microbiol Infect Dis 1980; 1:259-267.
14. **WOODWARD LF, RENSHAW HW, BURGER D, McCAIN CS, WILSON RB.** Cell-mediated immune responses of neonatal calves and adult cattle following innoculation with PPD of *Mycobacterium bovis* associated with a mycobacterial immunopotentiating glycolipid and oil droplets. Am J Vet Res 1979; 40:636-644.
15. **WOODWARD LF, RENSHAW HW, BURGER D.** Onset, kinetics and duration

of *in vivo* and *in vitro* delayed hypersensitivity responses of neonatal calves sensitized with mycobacterial components. Vet Microbiol 1979; 4:169-176.

16. **ROSSI CR, KIESEL GK, HUDSON RS, POWE TA, FISHER LF**. Evidence for suppression or incomplete maturation of cell-mediated immunity in neonatal calves as determined by delayed-type hypersensitivity responses. Am J Vet Res 1981; 42:1369-1370.
17. **SCHOONDERWOERD M, DOIGE CE, WOBESER GA, NAYLOR JM**. Protein energy malnutrition and fat mobilization in neonatal calves. Can Vet J 1986; 27:365-371.
18. **PURTILO DT, CONNOR DH**. Fatal infections in protein-calorie malnourished children with thymolymphatic atrophy. Arch Dis Child 1975; 50:149-156.
19. **CHANDRA RK, GUPTA S, SINGH S**. Inducer and suppressor T cell subsets in protein-energy malnutrition. Analysis by monoclonal antibodies. Nutr Res 1982; 2:21-26.
20. **CHANDRA RK**. Numerical and functional deficiency of T helper cells in protein-energy malnutrition. Clin Exp Immunol 1983; 51:126-132.
21. **BAKER PE, KNOBLOCK KF**. Bovine costimulator. II. Generation and maintenance of a bovine costimulator-dependent bovine lymphoblastoid cell line. Vet Immunol Immunopathol 1982; 3:381-397.
22. **DAVIS B, HUFFMAN M, KNOBLOCK K**. Logit analysis of interleukin 2 (IL 2) activity using a basic program. Comp Applic Lab 1983; 1:269-275.
23. **KWAPINSKI JB**. Methods of serological research. New York: John Wiley and Sons, Inc., 1965: 210-217.
24. **DANIEL WW**. Applied nonparametric statistics. Boston: Houghton Mifflin Company, 1978.
25. **PURI S, CHANDRA RK**. Nutrition regulation of host resistance and predictive value of immunologic tests in assessment of outcome. Pediatr Clin North Am 1985; 32: 499-516.
26. **BLOOD DC, RADOSTITS OM, HENDERSON JA**. Veterinary medicine. A textbook of cattle, sheep, pigs, goats and horses. 6th ed. London: Baillière Tindall, 1983: 754.
27. **CABELLO G**. Neonatal changes in the plasma level of cortisol, cortisone and aldosterone in the calf. Biol Neonate 1979; 36:35-39.
28. **EBERHART RJ, PRATT MS**. Plasma cortisol concentrations in newborn calves. Am J Vet Res 1971; 32:1921-1927.
29. **BLECHA F, BAKER PE**. Effect of cortisol *in vitro* and *in vivo* on production of bovine interleukin 2. Am J Vet Res 1986; 47:841-845.
30. **GILLIS S, CRABTREE GR, SMITH KA**. Glucocorticoid-induced inhibition of T-cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. J Immunol 1979; 123:1624-1638.
31. **AKANA SF, CASCIO CS, SHINSAKO J, DALLMAN MF**. Corticosterone: narrow range required for normal body and thymus weight and ACTH. Am J Physiol 1985; 249:R527-R532.
32. **ROOSNEK EE, BROUWER MC, AARDEN LA**. T cell triggering by lectins. II. Stimuli for induction of interleukin 2 responsiveness and interleukin 2 production differ only in quantitative aspects. Eur J Immunol 1985; 15:657-661.
33. **STUTMAN O**. Intrathymic and extrathymic T cell maturation. Immunol Rev 1978; 42:138-184.
34. **STUTMAN O**. Two main features of T cell development: thymus traffic and postthymic maturation. Contemp Top Immunobiol 1977; 7:1-46.
35. **DAUBENER W, VON STELDERN D, MAXEINER B, SCHEURICH P, PFIZENMAIER K**. Postnatal development of functional T cell subsets in the mouse: a frequency analysis of mitogen reactive precursors of proliferating, of cytotoxic and of IL2 producing T cells. Immunobiology 1985; 169:472-484.
36. **FOSSUM C, LARRSON B, MATSSON P**. Development of mononuclear cell subpopulations and their functions during calfhood. J Vet Med B 1986; 33:518-527.
37. **ROIT I, BROSTOFF J, MALE D**. Regulation of the immune response. In: Immunology. Toronto: The C.V. Mosby Company, 1985: 10.1-10.12.