This is to certify that the

thesis entitled

Influence of dietary vitamin E on health and humoral immunity of neonatal calves

presented by

Judith Viola Marteniuk

has been accepted towards fulfillment
of the requirements for

M. S. degree in Large Animal Clinical Sciences

Howard D. Stowe D.V.M.
Major professor Ph.D.

Date October 31, 1984

INFLUENCE OF DIETARY VITAMIN A ON HEALTH AND HUMORAL IMMUNITY OF NEONATAL CALVES

By

Judith Viola Marteniuk

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Large Animal Clinical Sciences

1984

ABSTRACT

INFLUENCE OF DIETARY VITAMIN A ON HEALTH AND HUMORAL IMMUNITY OF NEONATAL CALVES

By

Judith Viola Marteniuk

Two experiments involving 25 neonatal Holstein calves were conducted to determine: 1) the importance of dietary vitamin A to the health of neonatal calves; 2) the serum vitamin A levels of calves fed different dietary levels of vitamin A; and 3) the influence of vitamin A on humoral immune parameters in calves. The calves were fed a pooled vitamin A-low colostrum with three levels of retinyl palmitate supplementation (0.5, 1.0, 3.0 times NRC-proposed vitamin A requirements). Serum samples were obtained and analyzed for vitamin A, immunoglobulins and total protein. Body weights and health of calves were monitored. Calves fed 1.0 and 3.0 times NRC vitamin A were healthier than calves fed 0.5 NRC vitamin A. All calves fed 0.5 NRC vitamin A in Experiment 1 died by seven days of age; however, in Experiment 2 all calves survived but exhibited a greater incidence of pneumonia and diarrhea than calves in other groups. No significant difference in body weight, IgA, IgM or total serum protein were obtained. However, after brucella vaccination, there was a significant positive relationship ($P<0.1$) between IgG and dietary vitamin A supplementation levels. Serum vitamin A levels were not

significantly different between the surviving calf groups in Experiment 1; however, in Experiment 2 the serum vitamin A of the 3.0 times NRC group was significantly greater than either of the other two groups.

ACKNOWLEDGEMENTS

Sincere thanks are extended to Dr. H. Stowe for all his patience and assistance in making the completion of my Masters of Science Degree possible. Thanks are also extended to my committee, Drs. R. Emery, T. Herdt, R. Patterson, and D. Ullrey for their counsel.

Special thanks are also extended my husband, Kent and to the Tousley family for their patience, assistance and encouragement for without their help this thesis would not be a reality .

I also wish to thank all my friends and colleages who patiently endured during the writing of my thesis.

TABLE OF CONTENTS

LIST OF TABLES

page

TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Calf mortality represents a tremendous loss to both the dairy and beef industry. Mortality ranges from 8-25% annually with an economic loss in excess of $200 million/year. Further economic losses are experienced by the cattle industry due to dcreased productivity of recovered individuals, however, this economic loss is hard to estimate

The calf mortality occurs within the first 2 months of life and is ultimately a result of respiratory or digestive disease. Although infectious agents such as E. coli and viruses (rota and corona) receive the primary emphasis, other factors such as nutrition, immunity, environment and management must be considered.

As the calf is aggamalobulinemic at birth and relies on colostrum for its initial immunity, the importance of early colostrum ingestion to calf survival is well documented. However, the calf is also born deficient of the fat-soluble vitamins. The importance of the high-fat soluble vitamin content of colostrum to neonatal survival and immunity is poorly understood. Recent studies in other species have demonstrated a role between vitamin A supplementation and immunity.

The objectives of the following research were to determine: 1) the importance of vitamin A to health of neonatal calves, 2) the relationships between dietary and serum vitamin A levels, and 3) the influence of dietary vitamin A on humoral immune status.

LITERATURE REVIEW

VITAMIN A

Background

A vitamin is defined as an organic substance which the body requires in small amounts for its metabolism, yet cannot make for itself, at least in sufficient quantities, from proteins, carbohydrates or fats (Rollins 19). In 1913, McCollum and Davis reported the existence of an essential lipid-soluble substance which they named fat-soluble A (later called just vitamin A) that was capable of promoting growth in rats. By 1930-1931, Karrer and associates had determined the chemical structures of beta-carotene and retinol (vitamin A). With the determination of these structures, the relationship between the carotenes of plants and vitamin A of animals was clarified. Vitamin A is a generic term used to group all compounds that exhibit the biological activity of retinol. However, more recently, the term retinoids is the term being used. Both the natural forms of vitamin A and the synthetic analogs, which may or may not have the biological activity of retinol, are included in the retinoid grouping.

Function, Metabolism and Storage

Good reviews on vitamin A function, metabolism and storage are presented by Goodman (1980) and Olson (1969). Natural sources of vitamin A include the plant carotenoids and the long-chain retinyl esters of animal tissues. The carotenoids (primarily beta-carotene) are converted to retinol in the intestinal mucosa by two enzymes, beta carotene-15-15′ dioxygenase and retinaldehyde reductase. Dietary retinyl esters are hydrolyzed in the small intestine and retinol is absorbed into the mucosal cell. Bile salts are required for the reaction to occur. Once in the intestinal mucosal cell, retinol is reesterified with long chain fatty acids and the retinyl esters are absorbed into the body. The retinyl esters are then transported to the liver via the lymph chylomicrons. After uptake by the liver parenchymal cells, the retinyl esters undergo hydrolysis and reesterification. The resulting retinyl esters (mainly retinyl palmitate) are stored in association with lipid droplets. Vitamin A is mobilized from the parenchymal cells by hydrolysis of the retinyl esters to retinol. The retinol is then bound to retinol-binding protein (RBP) which is the specific plasma transport protein for retinol. Retinol-binding protein (RBP) is synthesized in the liver and, in circulation, interacts strongly with prealbumin to form a 1:1 molar protein: protein complex.

At the target tissue, retinol is associated with cytoplasmic retinol binding protein (CRBP) which is a distinct protein form plasma RBP. The way vitamin A functions in a cell is not specifically known. However, it has been proposed that CRBP and cytoplasmic retinoic acid binding protein (CRBP) may play a direct role in the biological expression of vitamin A. Another possibility is that CRABP and CRBP specifically direct the retinoids from one area to another within the cell (Chytil and Ong 1978).

Although retinol is the main biologically active retinoid, retinoic acid does exhibit selective biological activity. Retinoic acid is unable to support reproduction or prevent blindness due to vitamin A deficiency. Transportation of retinoic acid to the liver is through the portal system bound to serum albumin rather than through the lymphatic system. Retinoic acid is not stored in any of the body tissues in appreciable amounts, hence very little retinoic acid is present in the body.

The distributions of CRABP and CRBP are different. Cytoplasmic retinol binding protein is widely distributed and has been present in all adult tissues studied except serum and muscle. On the other hand, CRABP is absent from the kidney, small intestine, liver, lung and spleen, as well as muscle and serum.

Observations involving perinatal tissue levels of CRABP and CRBP suggest these proteins may be involved in the changing utilization of retinol and retinoic acid by various tissues. Cytoplasmic retinoic acid binding protein is present in all perinatal tissues except serum and disappears from the various tissues during certain developmental changes. There appears to be no obvious association between the tissue levels of CRBP and CRABP or the tissue level changes in CRBP and CRABP that occur during perinatal development, thus suggesting that the binding proteins are regulated independently (Chytil and Ong 1978). Excretion of Vitamin A is premarily through the urine as retinoic acid. However, some is excreted through the bile in the form of retinyl beta-glucuronide.

Deficiency, Toxicity and Requirements

Vitamin A deficiency may occur in the livestock industry today for a wide variety of reasons. The diet may be deficient due to wide variations in the vitamin A content of feedstuffs, vitamin A antagonists (e.g., vitamin E, Ca,S, Mn and I) may be present in the diet, and least-cost diet formulations may have reduced or exluded certain vitamin preparations. The above problems may be potentiated by the use of restricted feeding programs where livestock are fed stored feeds (concentrates and roughages) and little or no pasture (Hibbs 1980; Rollins 19).

Certain diseases and other stressful situations also increase the body's demand for vitamin A. Genetic selection for high-producing cattle increases the dietary requirements thereby increasing the chances of deficiencies occurring. The earliest detectable change in a vitamin A deficiency (30-33 ug beta-carotene/454 g body weight/day) is an increase in cerebral spinal fluid (CSF) pressure; if deficiency is more severe (11-16 ug beta carotene/454 g body weight/day) individuals exhibit increased lacrimation and night blindness which progress to xerophthalmia and blindness (Eaton 1969; DeLuca 1969). In severe deficiency, convulsions, anorexia and weight loss occur; the hair coat becomes rough and scaly; respiratory and digestive problems increase; and lameness and swelling of joints and brisket and reproductive disorders occur (Hibbs 1980; Vasudevan and Dutt 1969). If severe deficiency occurs in nature individuals (both male and female), complete failure of reproduction occurs. If deficiency is not as severe, only early embryonic death or birth of weak or dead fetuses occurs. Young individuals show the most rapid and severe signs when a vitamin A-deficient diet is fed. If severe enough, death often occurs within the first week of life (Chew and Archer 1983).

The ability of vitamin A to control and direct differentiation of epithelial tissues was established early

in the 20th century by Wolacn and Howe (1925) with studies involving rats fed diets deficient in vitamin A and then resupplemented with vitamin A. In vitamin A deficiency, the mucous-secreting lining of epithelial tissues is replaced by a squamous metaplastic epithelium, which eventually produces large amounts of keratin. The one exception is the intestinal mucosa where there is only a significant decrease in goblet cells.

The epithelial tissue changes that occur during vitamin A deficiency can be reversed by vitamin A supplementation and in vitamin A toxicity, normal keratinized epithelial tissues may be induced to mucous secretion. Other changes which occur during vitamin A deficiency or toxicity are abnormal bone and cartilage remodelling due to abnormal osteoblast/osteoclast distribution and function. Vitamin A toxicity results in anorexia, weight loss, fatigue and hepatomegaly (DeLuca 1977).

At the cellular level, some glycoprotein biosynthesis is decreased in vitamin A deficiency and enhanced by excessive vitamin A. Vitamin A may act as the lipid portion of a glycolipid intermediate during certain glycosylation reactions (DeLuca and Wolf 1969). This may explain some of the cellular changes seen in vitamin A-requiring tissues as glycoproteins are a major constitutent of cell membranes. The liver, which traditionally was considered as the primary

storage site for vitamin A, also undergoes biochemical changes in a deficiency. During vitamin A deficiency, hepatic conjugation of mannose virtually halts, RBP production is decreased, and RBP release to serum is blocked. Upon addition of vitamin A to the diet, RBP rapidly reappears in the serum. Thus, the net effect of vitamin A deficiency is an expended hepatic RBP pool (DeLuca 1977; Chytil and Ong 1978).

At the cellular level, vitamin A toxicity may result when the body's vitamin A level reaches a concentration that allows free retinol to circulate in plasma. This free retinol is then delivered to tissues in a nonspecific and unregulated manner in contrast to the highly regulated manner of delivery for RBP (Goodman 1980).

Dietary vitamin A is usually referred to in international units (IU) where 1 IU of vitamin A is equal to 0.3 ug of all trans-retinol or 0.334 ug of all trans retinyl acetate or 0.55 ug of all trans retinyl palmitate. The carotenoids are also related to retinol activity with 1 ug of retinol biologically equivalent to approximately 10 ug beta-carotene for cattle (NRC 1976).

Horses and ruminants are not as efficient as some species in converting beta-carotene to retinol. When compared to the rat, horses and cattle require four times as much beta-carotene to produce an equivalent amount of retinol.

The requirements for vitamin A vary depending on age and status of the individual and the vitamin A-dependent tissue considered. To prevent night blindness in cattle, approximately 17-26 IU of vitamin A, as retinol, per kg of body weight or 25-35 ug of beta-carotene/kg of body weight are required (Guilbert et al 1940). To sustain reproduction and allow liver storage, three times the above level of retinol or five times the above level of beta-carotene, are required.

Vitamin A requirements may also be expressed in terms of dietary intake. These requirements may be obtained by referring to the nutrient requirement publications of the National Academy of Sciences for the various species. If liver stores are adequate from a previously adequate diet (i.e., green forage) an animal may do well on low dietary levels of beta-carotene for periods of four to six months.

NUTRITION AND IMMUNITY

Immune System

General

Once the body's external barriers, (e.g., skin or gastrointestinal mucosa) have been penetrated, cells of the lymphoreticular system provide the main defense against foreign antigenic substances such as bacteria, viruses, and

vaccines. The major cells of the immune system are the lymphocytes. Lymphocytes are divided into two groups depending upon their fuction, membrane and physical properties, reactivity to various mitogens, and origin (Greaves et al 1974; Bach 1976). The T lymphocyte is derived from the thymus and is involved in cell-mediated immunity (CMI). Tne B lymphocyte is derived from the bone marrow and is involved in humoral immunity. Both B and T lymphocytes circulate intra- and extravascularly. They constantly distinguish if an antigen is foreign and react to that foreign antigen. When an antigen is trapped by phagocytic cells in tne lymphoreticular tissues and is presented to circulating immunocompetent B and T lymphycytes, a complex sequence of antigen-specific and non-specific cell reactions occurs (Beisel 1982) resulting in expression of CMI and/or humoral immune responses. Other ancillary circulating and cellular factors, which are not antigenically related to the primary infections, initiate other stimulatory or inhibitory changes in immune system functions. Also fever, phagocytic cell production and other non-specific responses may occur that are not related directly to antigenic properties of foreign agent (Suskind et al 1977).

Lymphoreticular tissues possess the highest rate of cell proliferation and thus experience increased nutritional

requirements over other body organs. If a decrease in nutrients occurs due to malnutrition or infection, the lymphoreticular tissues compete with other body organs for nutrients. If they are unable to obtain adequate nutrients, biochemical, metabolic and synthetic alterations occur.

Cell-Mediated Immunity

The T cells migrate from the thymus to the thymic-dependent areas of the lymphoreticular tissues, such as spleen and lymph nodes and are the major circulating lymphocytes. The T lymphocytes are the major cells of the CMI responses. The response of the T lymphocytes involves a complex sequence of interactions among T cells and of T cells with macrophages and with B cells (Swain 1980). The T cells are also capable of recognizing antigen. When an antigen attaches to a surface receptor of a T cell, the T cell proliferates and differentiates into T-secretory cells, T-helper cells, T-suppresor cells, T-killer cells and/or T-memory cells. The secretory cells release lymphokines which are the beneficial mediators of the CMI response (Cline 1975). Lymphokines are responsible for induction of uncommitted, immunocompetent T-cells, amplication of the induced cell, increased function of macropnages and phagocytes, and direct killing of target cells (Williams 1977). Cell mediated immunity is involved with delayed hypersensitivity reactions, graft rejections, and tumor

recognition and tumor immunity (Chandra 1972; Dutton 1980). Most investigators (Chandra 1972; Sellmeyer et al 1972; Beisel 1982; Rundles 1982) agree that protein calorie malnutrition (PCM) inhibits the CMI response, with the degree of inhibition proportional to the severity of PCM.

Humoral Immunity

The B lymphocytes are the major cells of the humoral immune response. The B cells migrate from the bone marrow to specific areas of the lymphoid tissue. The major function of the B cell is the production of immunoglobulins (Ig). The production of Ig is a complex process: B cells are controlled by T cells and require macrophages for antigen processing (Eardley 1980). Antigen is attached to surface receptors on the B cell leading to production of immunoglobulin and proliferation of the B cell into antigen-specific Ig effector cells (plasma cells) and memory cells (Williams et al 1977). Immunoglobulins are the mediators of the humoral immune response. Immunoglobulins bind to antigen, interact with phagocytic cells, promote antigen uptake and activate enhancing systems like the complement system. Different Ig predominate in different body fluids and at different concentrations and times in the immune response (Bernier 1978).

Immunoglobulins are multichained molecules comprised of two identical heavy and light chains linked by disulfide

bonding and monovalent forces. The caroxyl-terminal half (Fc portion) of the heavy chain determines the class specificity of Ig, e.g., complement activation (IgM and IgG) and binding to secretory piece (IgA). The amino-terminal quarter (Fab portion) of the heavy chain determines the specific antibody function and with the light chains provides the antigen combining site. Five classes of Ig are recognized: IgG, IgA, IgM, IgD and IgE. However, only IgG, IgA, and IgM have been recognized in bovine. The following is a summary of their locations and functions (Bernier 1978; Butler 1969).

1. IgG is found in the vascular and the extravascular spaces and is involved with complement, monocytes and neutrophils to promote phagocytosis of antigen. IgG, with four subclasses, is the most abundant Ig accounting for 85-90% of serum and colostral Ig.

2. IgA is the second most abundant Ig and is the first line of defense on mucosal surfaces (gut, respiratory and urinary). IgA activates the alternate pathway. Two subclasses of IgA have been identified. On mucosal surfaces, IgA is found in association with a glycoprotein, the secretory piece.

3. IgM is confined to the vascular space and is involved in the early response to an antigen. IgM is the only Ig normally present at birth. IgM also activates

complement. It is the largest Ig and accounts for 10% of serum and colostral Ig.

4. IgD may play a role in the expression and mediation of the immune response and is associated with the lymphocyte surface.

5. IgE mediates allergic responses by binding to mast cells and basophils causing degranulation and release of inflammatory mediators. IgE-producing cells are located in the same areas as IgA producing cells.

Nutritional Interactions

The statement "we are what we eat" is applicable to our immune system as well. The association of famine and disease has long been known (Axelrod 1971; Sellmeyer et al 1972; Shillhorn van Veen 1974; Chandra 1980; Wilgus 1980). When disease-nutrition interactions occur, the body's nutritional requirements are increased due to 1) the competition between host and invading organism for nutrients; 2) the increased activity of the immune system; 3) the decrease in absorption and utilization of nutrients; and 4) the increased excretion of nutrients (Scrimshaw 1977; Wilgus 1980). The above statements pertain to the four classes of nutrients: protein, energy, vitamins and minerals. Clinical kwashiorkor often occurs following an infection (e.g., diarrhea or measles) in young children who were previously suffering from a subclinical protein

deficiency. Marasmus is also potentiated by infections through lack of appetite and diet change during disease, as well as increased energy requirements. Protein-calorie malnutrition (PCM) is often considered together in the literature and has been the most frequent cause of acquired immune deficiency in man (Law et al 1973; Rundles 1982). Conversely, if the protein and energy intake are increased, children are healthier and have an increased antibody response (Mathews et al 1972; Reddy et al 1976; Suskind et al 1976). Cell mediated immunity and humoral immunity are both affected by PCM.

Vitamin deficiencies and infection also show a marked relationship (Scrimshaw 1977). Infective processes potentiate dietary vitamin deficiencies impairing the body's defense mechanisms to disease while increasing the body's vitamin requirements. Of the minerals, iron and zinc, have been shown to significantly affect the immune system (Rundles 1982).

The majority of relationships between infection and nutrition are synergistic, that is nutrient deficiencies increase the severity of infections. However, antagonistic relationships in which nutrient deficiencies impede infections do occur especially with viruses and protozoa that are intracellular and highly dependent on the host cell metabolism. The interaction between nutrition and immunity

is complex and may be direct or indirect. The former pertains to a direct effect of nutrients on the lymphoid system, while indirect pertains to an effect on cellular metabolism in general.

Vitamin A and Humoral Immuninty

The relationsnip of vitamin A to numoral immunity is not as well documented as with cell mediated immunity. In reviewing the literature, several variables were found to influence numoral immunity: age, species, nutritional status, form and amount of vitamin A and disease entity. Studies involving chickens have given the most consistent results with vitamin A deficienmcy associated with a decrease in weight of the thymus and depletion of lymphocytes and plasma cells from lymphoendothelial tissue (Bang et al 1972; Beisel 1982). Chickens have also shown a decreased resistance to bacteria (Alder and Da Massa 1972), protozoan and parasitic disease when vitamin A-deficient (Wilugs 1981). However, normal to excess vitamin A may increase the susceptibility of individuals to viral diseases and certain lymphoid tumors (Wilgus 1980). Others have shown vitamin A deficiency increased the incidence of colon and liver cancer (Wald et al 1980; Beisel 1982). Studies have also shown vitamin A supplementation may aid in treatment of certain tumors (Ong and Chytil 1983). Axelrod (1971) demonstrated a moderate decrease in antibody response

in the vitamin A-deficient white rat to human erythrocytes. Antibody response to diphtheria and tetanus toxoid in rats was also impaired in vitamin A deficiency (Beisel 1982). However, 200,000 IU vitamin A supplementation as a single dose produced no increase in antibody response in children in a Bangladesh study (Brown et al 1980). An increase in antibody response was demonstrated when 3000 IU vitamin A per day was given to mice. The mice also had a large increase in the number of splenic plaque-forming cells after inoculation with sheep erythrocytes.

Pletsityi (1982) has shown vitamin A supplementation increases immune response of patients with chronic pneumonia. Normal individuals showed no response to vitamin A supplementation. Pletsityi further demonstrated an increase in the weight of lymphoid organs and an increase in serum antibodies when exposed to certain antigens. Vitamin A may exert its effect on the immune system by labilizing lysosomes and stimulating cell division and phagocytosis (Allison and Mallucci 1964; Roels 1969 and Jurin and Tannock 1972). Further, it has been proposed that vitamin A is required in glycoprotein synthesis, hence vitamin A may be involved with cell membrane formation and stability and immunoglobulin synthesis (DeLuca and Wolf 1969; Bohannon et al 1979).

Not all immune mechanisms are beneficial. Some detrimental occurrences are serum sickness or anaphylactic reactions, formation of antigen-antibody complexes and their precipitation in various tissues, graft rejections and initiation of cell-destroying reactions in host tissues associated with postinfection complications (Beisel 1980; Dutton 1980).

THE CALF

Mortality and Economics

One of the greatest losses to the cattle industry occurs in the preweaned calf. Amstutz (1965) reported that 8-25% of all calves die annually of intestinal disease. This converts to an economic loss of $50 million/year. No estimation of the economic loss due to decreased productivity of surviving individuals was made. Also, calving problems and respiratory disease would further increase the economic loss. Martin (1975) reported calf mortality in Tulare county, California was approximately 20% (3.7% to 32.1%) in 16 herds. Oxender et al (1979), reported calf mortality in Michigan was approximately 18%. This mortality rate converts to an economic loss in the 70's of over $200 million/year. Of the death losses occuring before calves are weaned, 55% occurs within one week of age and 80% occurs within two weeks of age. The mortality tends to be

seasonal with the mortality being highest in mid-summer and mid-winter.

Role of Colostrum

The importance of colostrum has been known since 1922. The epitheliochorial placenta of the cow does not allow immunoglobulin transfer across the placenta to the fetal calf; hence, all immunoglobulins obtained by the calf are through colostrum ingestion. The absorption of colostrum by the neonatal calf is a non-selective process during which B and T cells, macrophages, bacteria, other proteins, vitamins and other constituents in addition to immunoglobulins are transferred into the mucosal cell. The importance of the colostral constituents to the health of the calf is unknown. Some specificity is exerted by the intestinal mucosal cell as to constituents entering the lymphatic system.

Absorption of colostrum declines rapidly after birth with gut closure being complete between 25 and 48 hours. Gut closure is a gradual retrograde response. The mucosal cells' basal membrane ceases to release colostral components into the lymphatics. Upon gut closure, cellular transport then ceases and eventually uptake of colostral components by the tubular system ceases (Bush and Staley 1980; Clover and Zarkower 1980).

Colostral absorption by the newborn calf is affected by numerous entities. Actual ingestion of colostrum by the

calf is of primary concern. If the calf is left with the cow, colostral ingestion is affected by the dam's production of colostrum and mothering ability, by the shape, placement and size of the udder and by the vigor and drive of the calf to suckle. The calf's suckling drive and vigor are affected by dystocia, genetics and ambient temperature. Because of the phenomena associated with the process of gut closure, the calf must suckle or be fed as soon as possible after birth, preferably within two hours to maximize the absorption of colostrum. The quality and quantity of colostrum ingested also markedly affects colostral absorption.

The quality and quantity of colostrum is affected by the genetics, age, immune status and nutrition of the dam (Bull et al 1974); Selman et al 1971; Stott et al 1979; Brignole and Stott 1980). Quality of colostrum may be estimated by measuring its specific gravity (Fleenor and Stott 1980). Calves absorb more immunoglobulins when fed more colostrum at the first feeding rather than when colostrum is split into two equal feedings at 12 hour intervals (Stott et al 1979). Colostrum absorption is also greater when calves ingest frozen or fresh colostrum rather than fermented colostrum (Bush and Staley 1980). Although absorption of fermented colostrum may be improved by adjusting the pH to near 7, absorption is still less than

when fresh or frozen colostrum is fed. Only first-milking colostrum should be fed as immunoglobulins decrease by one-half during the first 12 hours.

Heat stress or separation from the dam also decreases colostrum absorption (Stott et al 1980). Corticosteroid injections and endogenous cortisol do not interfere with the calf's ability to absorb colostrum, as is seen in other species (Bush and Staley 1980; Bush et al 1980). Corticosteroids may even enhance absorption of colostral immunoglobulins by the calf (Johnson and Oxender 1979). However, administration of slow-release corticosteroids to the cow does inhibit absorption of IgG (Stott et al 1980). Although there are conflicting reports (Olson et al 1980), most researchers agree that depriving the cow of dietary protein also tends to decrease immunoglobulin absorption by the calf. Genetics within and between breeds may also play a role. The quality of colostrum ingested may also affect absorption of individual immunoglobulins with IgM and possibly IgA being absorbed more efficiently at low colostral intakes (Bush and Staley 1980).

Colostrum has a local protective effect in the gut as well as the systemic effect associated with absorption (Logan et al 1974). Studies have shown that, although gut closure occurs during the first 24 to 48 hours and immunoglobulin absorption is markedly decreased by 12 to 24

hours, calf mortality can be significantly decreased by feeding colostrum for at least three days. It is unknown if the local protective effect of colostrum is due only to the immunoglobulin content or if other constituents of colostrum play a role (Selman et al 1970; Brignole and Stott 1980).

Daily Nutritional Requirements

Calves should receive 10% of their body weight per day of both colostrum and milk or milk replacer. Adequate immunoglobulins for the average calf are provided by 3.25 liters of colostrum with a whey immunoglobulin concentration of 80 mg/ml (Fleenor and Stott 1980). After the third day of feeding colostrum, the calf may be maintained on whole milk or milk replacer. Whole milk is traditionally the superior diet following colostrum. If a milk replacer is fed, only one of good quality should be used due to the neonatal calves' inability to digest certain nutrients when less than two weeks of age. The milk replacer should contain 22-24% of high quality, milk-derived protein, at least 10% fat and a fiber content of not more than 0.5%. If calves are to be housed outside during the winter, a milk replacer with 20% fat content should be used.

Calves should be introduced to calf starter and good quality hay within 1 week of birth. After absorption of colostral vitamin A, the daily vitamin A intake of the calf should be approximately 2000 IU. This is easily met by milk

obtained from cows on a normal vitamin A intake. However, if the diet of the cow is not supplemented, and the cow is receiving a stored feed, the vitamin A intake by the calf may be deficient. Milk replacers usually contain high levels of vitamin A, approximately 44,000 IU per kg of dry matter.

Immune Status

As previously stated, the neonatal calf relies on colostrum for its early immunity (Clover and Zarkower 1980). However, many calves are hypo- or agammaglobulinemic due to failure of passive transfer or failure to ingest adequate colostrum before gut closure. One study indicated up to one-third of all calves that suckle may still be agammaglobulinemic (Brignole and Stott 1980). After colostral ingestion, the immunoglobulin levels should be 11-15 mg/ml of blood (Bull et al 1974). If IgG levels are less than or equal to 21.0 mg/ml calves usually die, however, if IgG levels are equal to or greater than 6 mg/ml calves usually survive (Penale et al 1973).

Recent evidence indicates that the bovine fetus is immunocompetent from four months of gestation (Smith and Ingram 1965; Horyna et al 1980; McGuire and Adams 1982). Thus, calves are born immunocompetent and agammaglobulinemic. If they fail to absorb colostral immunoglobulins and remain agammaglobulinemic,

immunoglobulin synthesis can be detected within a few days of birth. In hypogammaglobulinemic calves, immunoglobulin synthesis can be detected by 14 days of age (Bush and Staley 1980). Maternal antibodies tend to suppress the calves' immune response. Calves with normal immunoglobulin due to colostral ingestion do not begin significant immunoglobulin synthesis until approximately 4 weeks postpartum (Logan et al 1973).

Normally, immunoglobulin levels in serum peak 24 hours postpartum, drop at 2-4 weeks of age and then, due to immunoglobulin synthesis, begin to rise again (Vajda and Slanina 1980). Attemps to measure the potential of the neonatal calf's immune system has produced varying reports depending on antigens studied, route of antigenic challenge, neonatal age and circulating maternal antibodies (Clover and Zarkower 1980).

Selection

Investigation of the relationship between vitamin A and the humoral immune system of the neonatal calf was conducted for a number of reasons. As the calf is born vitamin A-deficient and relies on colostrum for its vitamin A stores (Hansen et al 1946; and Radostitis and Bell 1970), vitamin A-deficient calves for study should be easily obtained. Also, others have shown that, in certain species vitamin A enhances humoral immunity with regard to certain

diseases/immunizations (Cohen and Cohen 1973; Leutskay and Fair 1977; Tengerdy and Brown 1977; Brown et al 1980; Pletsity et al 1982). No information is available with regard to the bovine. Finally, because of economic loss in the cattle industry yearly due to neonatal mortality, the potential for vitamin A to enhance the immune status of the neonatal calf needs further investigation.

MATERIALS AND METHODS

NUTRITION

Colostrum

Colostrum was obtained from Holstein cows at the Michigan State University (MSU) dairy herd and frozen. When a sufficient volume of colostrum had been collected for these experiments, it was thawed and pooled. In order to obtain a colostrum deficient in vitamin A, the colostrum was then heated to 42 C and passed through a cream separator. The cream fraction containing the fat-soluble vitamins was discarded. The skimmed colostral fraction was then divided into 25 aliquots of approximately 3.7 liters each and refrozen. The skimmed fraction of colostrum was determined to contain 0.57 IU of vitamin A/ml. This is in contrast to whole colostrum which contains approximately 10 IU of vitamin A/ml (5 - 30 IU/ml).

An animal fat supplement[1] was obtained as a low-vitamin-A-fat replacement for the skimmed colostrum (Appendix 3). This fat supplement was added to the thawed, skimmed, pooled colostrum at 0.5 kg/3.7 liters to recreate a colostrum containing approximately 6% fat and minimal

---

1. Land O' Lakes, Webster City, IA 50595.

Figure 1

Diagramatic representation of the preparation of the colostrum treatments used in Experiments 1 and 2.

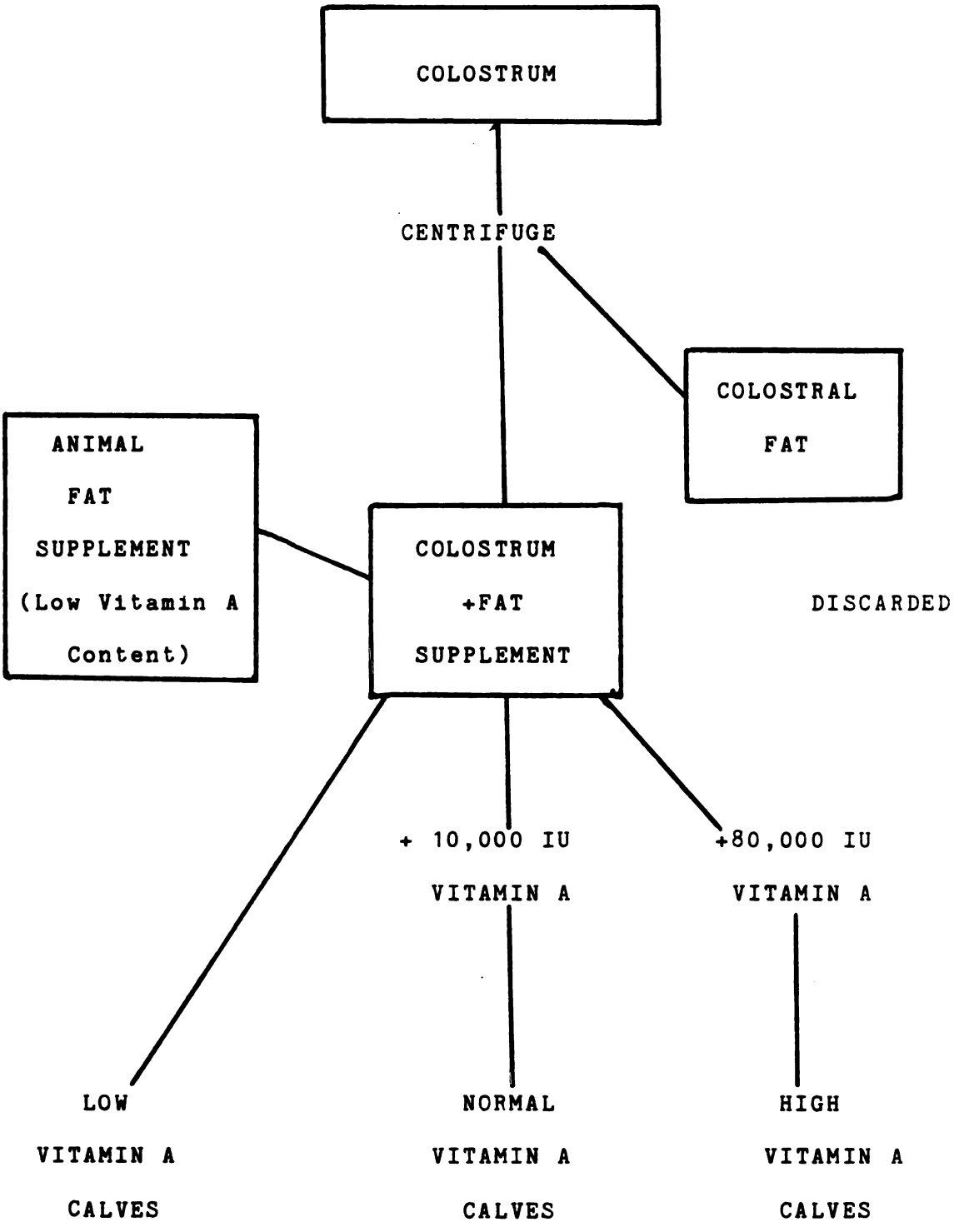
COLOSTRUM
CENTRIFUGE
COLOSTRAL FAT
ANIMAL FAT SUPPLEMENT (Low Vitamin A Content)
COLOSTRUM +FAT SUPPLEMENT
DISCARDED
+ 10,000 IU VITAMIN A
+80,000 IU VITAMIN A
LOW VITAMIN A CALVES
NORMAL VITAMIN A CALVES
HIGH VITAMIN A CALVES

Figure 1.

Figure 2

Diagramatic representation of the preparation of the diet for Experiments 1 and 2.

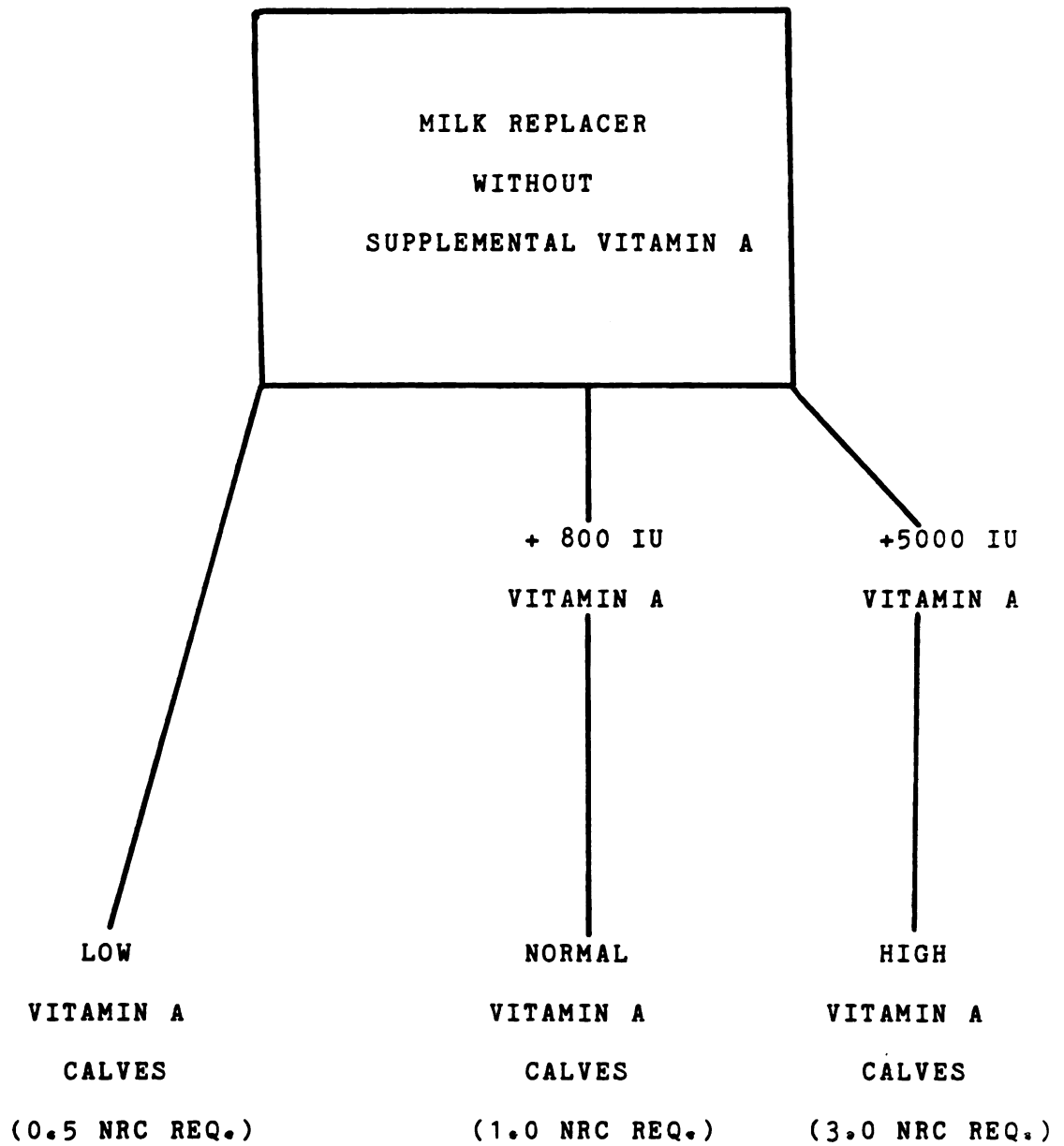
MILK REPLACER
WITHOUT
SUPPLEMENTAL VITAMIN A
+ 800 IU
VITAMIN A
+5000 IU
VITAMIN A
LOW
VITAMIN A
CALVES
(0.5 NRC REQ.)
NORMAL
VITAMIN A
CALVES
(1.0 NRC REQ.)
HIGH
VITAMIN A
CALVES
(3.0 NRC REQ.)

Figure 2.

vitamin A.

The calves were randomly allotted to one of three vitamin A supplementation levels. Each calf on experiment received only this reconstituted colostrum at approximately 10% of its body weight in two equally divided feedings during its first day of life (Figure 1).

Milk Replacer

The calves were maintained on a specially prepared milk replacer which contained only milk products (Appendix 2). The formulation was a commercial milk replacer[2] with the exception that no supplemental vitamin A had been added. The vitamin A content of the experimental milk replacer was 4.3 IU/gm in contrast to the normal commercial milk replacer which contained 66 IU of vitamin A/gm.

A feeding schedule[3] was followed to allow calves to be fed exclusively milk replacer for the entire study period and still have a positive weight gain, thus eliminating the need for multiple vitamin A-deficient rations to be formulated (Appendix 1). This schedule was followed as long as the calves remained healthy. If a calf developed diarrhea, the ration was decreased to the previous level and

---

2.Ñ Land O' Lakes, Webster City, IA 50595.

3.Ñ Land O' Lakes, Webster City, IA 50595.

maintained until the calf no longer had diarrhea. The ration was then increased according to the appended feeding schedule; however, the calf would now be at a lower level of intake than was listed for its age. After the fourth week of the experiment, calves were held at the 28-day intake level until the experiment ended.

Vitamin A Supplementation

The calves were randomly allotted to one of three vitamin A supplementation levels. Vitamin A was added to both the colostrum and milk replacer using a water-dispersible retinyl palmitate[4]. The retinyl palmitate was weighed twice weekly, mixed with water and refrigerated until used. The colostrum was supplemented at three levels of vitamin A (0, 10,000 and 80,000 IU/3.7 L). The vitamin A supplement was added at the first colostral feeding. Calves were continued on a daily vitamin A supplementation in the milk replacer at 0, 800 IU/day, and 5000 IU/day, respectively (Figure 2).

The daily supplemented and naturally present vitamin A in the diet resulted in calves receiving 0.5, 1.0 and 3.0 times the National Research Council (NRC)-proposed requirement for vitamin A.

4.N Sigma Chemical Co., St. Louis, MO 63178.

Whole Milk

Due to health problems encountered with calves shortly after initation of Experiment 1, a fourth experimental group was added. The calves in this group were handled as herd replacement calves for MSU. Calves were fed dam's colostrum for four feedings, fed whole milk until weaning, and offered hay and calf starter free choice within one week after birth.

Animals

Twenty-five Holstein calves were used in Experiments 1 and 2. Calves were obtained before colostrum ingestion and fed as previously described. Three ml of a vitamin E-Se preparation[5] were given subcutaneously at birth to all calves. Sixteen calves were used in Experiment 1. The calves were obtained from the MSU dairy herd and no attempt was made to standardize calves as to weight, sex or parity of the dam. Four calves were randomly assigned to each of four feeding groups: 0.5, 1.0, 3.0 times NRC-proposed vitamin A requirement and whole milk.

Nine calves were used in Experiment 2. The calves were obtained from a large local dairy[6] and were standardized as

---

5.N Burns biotec Laboratories, Inc.,Omaha, NE 68127.

6.N Green Meadows Farm, Ovid, MI 48866.

much as possible in that only male calves that weighed approximately 40-50 kilograms and were from at least second-parity cows were selected. Calves were to be born unassisted in normal presentation and were to be alert and vigorous. Three calves were assigned to each of three feeding groups: 0.5, 1.0 and 3.0 NRC-proposed vitamin A requirement.

Housing

In Experiment 1, calves were housed in calf hutches used for the MSU herd replacements. The hutches were constructed of plywood and measured 1.2 x 1.2 x 2.4 meters. A drop-down panel at the back of hutch allowed access for feeding. Hutches were placed in two rows on a hill facing south. The temperature varied greatly as the experiment was conducted in December, 1979 and January, 1980.

In Experiment 2, calves were housed in box stalls in Barn J at the Veterinary Research Farm. One stall was used for each experimental group (three calves per stall). Calves were tied to prevent contact between the calves. Temperature in the stalls was maintained at approximately 20 C.

Handling of Calves (Experiments 1 and 2)

All calves were weighed at birth and then at weekly intervals until the termination of the experiments. Blood samples were collected via the jugular vein from calves

Figure 3

Scoring index to record the daily health of the calves for Experiments 1 and 2.

SCORING INDEX FOR HEALTH OF CALVES

0 = Death

1 = Near death, severe denydration

2 = Severe diarrhea, severe respiratory disease, on treatment

3 = Slight diarrhea, slight respiratory disease

4 = Slight depression or attitude change

5 = Healthy

Figure 3.

precolostrum, 24 hours postcolostrum, and at weekly intervals until 4-5 weeks of age. At 4-5 weeks of age, all calves received one-fourth dose brucella vaccine[7]. Brucella vaccine was selected because of its immune-stimulating ability for both cellular and humoral immune systems (Cunningham 1977). Blood samples were then obtained from the calves at 3 and 7 days post-brucella vaccination. All blood samples were allowed to clot and then immediately centrifuged and the serum removed and stored frozen in disposable plastic vials[8]. Serum was analyzed for total vitamin A content (retinyl palmitate, retinyl acetate, and retinol), total protein and immunoglobulins (IgM, IgA, IgG). Health of the calves was monitored daily and scored. The scoring ranged from 0 through 5 (Figure 3).

Analysis of Samples

High pressure liquid chromatography (HPLC) was used for vitamin A analysis (Stowe 1982). One ml of sample (serum, milk replacer, colostrum) was combined with 1 ml of absolute ethanol in a disposable test tube and vortexed to denature the protein. Two ml of hexane (68 to 69 degrees C) were then added to each tube and vortexed for 1 minute. Samples

---

7.Ñ Jensal, Jensen Salsbery Laboratories, Kansas City, MO 64141.

8.Ñ Walter Sarstedt, Inc., Princeton, NJ 08540.

were then centrifuged for 10 minutes at 3000 rpm. The hexane layer was removed and passed through a .45 um millipore filter[9] in a swinny-type filter holder. One hundred ul of each extract were then injected into the HPLC system[10]. Separation was achieved using a microporasil column (39 mm ID and 30 cm long). The mobile solvent was a 60:40 mixture of degassed and filtered hexane and chloroform pumped at 2.5 ml/minute at 63.4 kg/cm2 pressure. A spectrofluorometer[11] equipped with a 35-ul flow cell and set at 330 and 470 nm for excitation and emission wave lengths, respectively, was used for detection of the 3 forms of vitamin A. The retention times for retinyl palmitate, retinyl acetate and retinol on the column were 82, 94, and 414 seconds, respectively. An integrating recorder[12]

---

9. Millipore Corporation, Waters Association, Inc., Milford, MA 01757.

10. M600 pump and H6K injector, Waters Association, Inc., Milford, MA 01757.

11. Aminco (J-4-8960) spectroflurometer, Silver Springs, MD 20910.

12. Model 730 Data Module, Waters Association, Inc., Milford, MA 016757.

was used to display vitamin A peaks and calculate vitamin A concentrations in the respective samples.

The above method was used to determine the inherent vitamin A content of the skimmed colostrum, calf milk replacer, and fat supplement and to determine the actual amount of vitamin A supplementation via colostral supplementation and daily milk replacer supplementation with retinyl palmitate.

Radioimmunodiffusion plates[13] were used to determine the IgG, IgA, IgM concentrations in the serum samples. The plates, standards and serum samples were allowed to reach room temperature. Upon reaching room temperature, a set volume of standard or serum was placed in the wells: 2.5 ul, 10.0 ul, and 10.0 ul for IgG, IgA, and IgM determination, respectively. Each box contained 3 plates and standards. Seventeen samples could be placed on each plate; however, the first four wells of row B on at least one plate per box were used for the 4 reference standards. After the wells were filled, a few drops of water were placed in the trough around the plate, the plates were covered, and left undisturbed at room temperature for 18, 22 and 22 hours for

---

13.N Radioimmunodiffusion plates, Miles Laboratories, Inc., Elkart, IN 46515.

IgG, IgA, and IgM, respectively. At the end of the respective incubation times, the diameters of the precipitation rings were measured directly from the calibrated immunodiffusion plate and recorded. A standard curve was then constructed by plotting the ring diameters of the standards on the x-axis against the known concentration of the standards on the y-axis using semilogarithmic graph paper. A straight line was then drawn to best fit the plotted data points.

The immunoglobulin concentrations of the samples were then obtained by directly reading points on the standard curve. The values recorded were equivalent to mg of immunoglobulin/100 ml of sample.

Total protein of the serum sample was obtained by using a refractometer[14]. A drop of serum was placed on the clean well. The refractometer was then held to the light and the total protein concentration was read directly. The well was cleaned between each sample with distilled water and cleaning tissues[15].

---

14. American Optical Company, Buffalo NY 14240.

15. Kim Wipes, Kimberly-Clark Corporation, Neenah, WI 54956.

Statistical Analysis

Data were analyzed using split-plot analysis of variance (Genstat) and multiple regression analysis (Sas).

RESULTS AND DISCUSSION

HEALTH OF THE CALVES

Health Scores

The health scores of calves in Experiment 1 are presented in Table 1. Ten of the sixteen calves survived. Of the six calves that died, four were from the dietary low vitamin A group (LA) and one from each of the high dietary vitamin A group (HA) and the whole milk group (WM). All six calves that succumbed in Experiment 1 died by one week of age as a result of diarrhea (scours) and dehydration. Of the ten calves that survived, those in the whole milk group were the healthiest. The normal dietary vitamin A (NA) group did well, but required more antibiotic therapy[16] than the WM group. The calves in the HA group experienced the most health problems and required the most treatment.

The health scores indicate that all calves were healthy at 24 hours of age; however, one week later, only one calf remained in the LA group and it was near death (died on day 7). One calf died early in the WM and HA groups (3 days and 7 days, respectively). The remaining calves in the HA and NA groups did exhibit respiratory and digestive disease.

---

16. Antibiotics used were penicillin, tetracycline, and chloramphenicol.

TABLE 1

MEAN HEALTH INDICES OF CALVES--EXPERIMENT 1

	Treatment group (n=4)			
	Low[a]	Normal	High[b]	Whole[c]
Calf Age	Vit. A	Vit. A	Vit. A	Milk
Precolostrum	4.9	5.0	4.9	5.0
Postcolostrum	5.0	4.8	5.0	4.9
1week	1.5	4.4	4.6	4.7
2 weeks	0	4.0	3.8	4.5
3 weeks	0	3.8	3.9	4.6
4 weeks	0	4.3	3.0	4.5
+3 dpv	0	3.3	2.0	5.0
+7 dpv	0	3.2	2.0	5.0

[a] 4 of 4 calves died by 1 week of age.

[b] 1 of 4 calves died at 1 week of age.

[c] 1 of 4 calves died before 1 week of age.

Mean health scores were obtained by averaging the daily health scores. See health scoring index (Figure 3).

dpv = days after brucella vaccination.

TABLE 2

MEAN HEALTH INDICES OF CALVES--EXPERIMENT 2

	Treatment group (n=3)		
Calf Age	Low Vit. A	Normal Vit. A	High Vit. A
Precolostrum	5.0	4.7	5.0
Postcolostrum	4.2	4.2	5.0
1 week	4.7	4.4	4.9
2 weeks	4.1	4.8	5.0
3 weeks	4.5	5.0	5.0
4 weeks	4.0	5.0	5.0
+3 dpv	3.8	4.9	5.0
+7 dpv	4.0	4.9	5.0

Mean health scores were obtained by averaging the daily health scores. See health scoring index (Figure 3).

dpv = days after brucella vaccination.

Although the calves often appeared normal, a decrease in health scores was recorded because the calves received antibiotic therapy.

The health scores of calves in Experiment 2 are presented in Table 2. The health scores again show all calves were healthy at birth. All calves survived the study; however, one calf from the LA group developed pyrexia, diarrhea and respiratory disease at three days of age and remained unthrifty throughout the entire study despite antibiotic therapy[17] . The lower scores reported for the LA group are mainly due to the one calf that was unthrifty, although all three calves had periods of pyrexia and diarrhea, especially after brucellosis vaccination. During the first day of life, calves from the NA group developed diarrhea. The diarrhea continued for approximately one week and then improved without treatment. All calves in the NA group did well. The low scores in the NA group, post colostrum ingestion and 1 week later, are due to the diarrhea these calves experienced early in the experiment. Calves in the HA group remained healtny throughout the study.

A number of factors may have contributed to the general poor health of calves in Experiment 1: 1) Environmental

---

17. Ñ Chloramphenicol.

conditions were poor during the winter of 1979-80 with many days of high humidity, increased precipitation (often as rain) and southerly winds (Appendix 4). 2) Housing was inadequate. Although calf hutches are an ideal means of housing neonatal calves, hutch construction is very important. It is essential that hutches are air tight on three sides to create a dead air space at the back thus preventing winds from blowing precipitation to the back of the hutches. Because these hutches had a drop-down rear panel that did not close tightly rain and snow were blown freely to the back of the hutch. Calves were often wet for several consecutive days due to inclement weather and wet bedding. 3) Hutch placement may also have been a factor. Some hutches were placed behind a stand of trees, while others were not sheltered to the south. Also a number of hutches were on a slope of a hill and, during rainy weather, often had water running beneath them. Placement of calves in the hutches was not random, and hutches of the HA group were placed in the poorest location. 4) Diet may have contributed to the health of the calves. As calves were maintained solely on a modified commercial milk replacer (Appendices 1 and 2) without hay or calf starter, they may not have received adequate energy under the inclement weather conditions even though the milk replacer contained 20% fat and the feeding schedule was constructed to provide

a positive energy balance and weight gain. It is believed that inadequate vitamin A played a significant role as all calves in the LA group died by seven days of age. Keener (1942) and DeLuca (1960) have shown that stress increases vitamin A requirements. Also, Chew (1983), reported that vitamin A-deficient neonatal rats died by 5 days of age. The WM group of calves may have been healthier than the other groups due to better location of hutches and the local protective effect of milk immunoglobulins (Brignole and Stott 1980).

Because of the inability to determine if the effect on health was a result of environmental conditions, housing conditions or a negative energy balance, the second experiment was conducted under more controlled housing conditions. All calves in Experiment 2 did better than calves in Experiment 1. The HA group had no health problems in contrast to Experiment 1. This tends to confirm that housing conditions played a large part in the poor health of the HA group in Experiment 1. Also, all deficient calves, although not as healthy as the calves in the other two groups, survived in Experiment 2. This may be a result of less stress and hence a lower vitamin A requirement for survival when calves are raised under controlled conditions (Keener et al 1942).

TABLE 3

WEIGHT GAIN OF CALVES FROM BIRTH TO 5-6 WEEKS OF AGE--EXPERIMENT 1

	Treatment group (n=4)			
	Low[a] Vit. A	Normal Vit. A	High[b] Vit. A	Whole[c] Milk
Replicate No.	(kg)	(kg)	(kg)	(kg)
1	----	12.27	6.82	12.27
2	----	5.91	----	----
3	----	14.09	4.09	15.00
4	----	9.55	6.82	4.55
Average weight gain	----	10.45 $\pm$3.56	5.91 $\pm$1.58	10.60 $\pm$5.42

[a] 4 of 4 calves died by 1 week of age.

[b] 1 of 4 calves died at 1 week of age.

[c] 1 of 4 calves died before 1 week of age.

TABLE 4

WEIGHT GAIN OF CALVES FROM BIRTH TO 5 WEEKS OF AGE--EXPERIMENT 2

	Treatment group (n=3)		
	Low	Normal	High
Replicate No.	Vit. A (kg)	Vit. A (kg)	Vit. A (kg)
1	13.60	15.91	15.91
2	14.09	16.82	15.91
3	-0.91	13.64	11.82
Average weight gain	8.96 ±8.52	15.45 ±1.64	14.55 ±2.36

Weight Gain

The weight gain of individual calves and the mean weight gain for the different dietary groups are represented in Tables 3 and 4. In Experiment 1, the HA group had the least amount of gain, while calves in the NA and WM groups had similar weight gains. These results may again reflect the environmental conditions, housing construction, and/or housing placement. This interpretation is supported by considering the individual and mean weight gains of the calves in Experiment 2. All calves, except the second calf in the LA group, gained well during the study period. Also, the fact that all calves (except LA-2), gained weight demonstrates that the milk replacer adequately met the energy requirements of the calves when housed under controlled conditions.

SERUM VITAMIN A

Retinyl Palmitate

The mean serum retinyl palmitate (rp) concentrations are represented in Figure 4 (Experiment 1) and in Figure 5 (Experiment 2). In Experiment 1, serum rp concentrations increased in all groups following colostrum consumption. The largest increase was in the WM group and the smallest increase was in the LA group. This was expected as the WM calves received a vitamin ADE injection and fresh colostrum

Figure 4

Serum retinyl palmitate concentrations of calves fed milk replacer with low, normal and high vitamin A content or whole milk. These data are from calves housed in calf hutches during the winter of 1979-80 (Experiment 1).

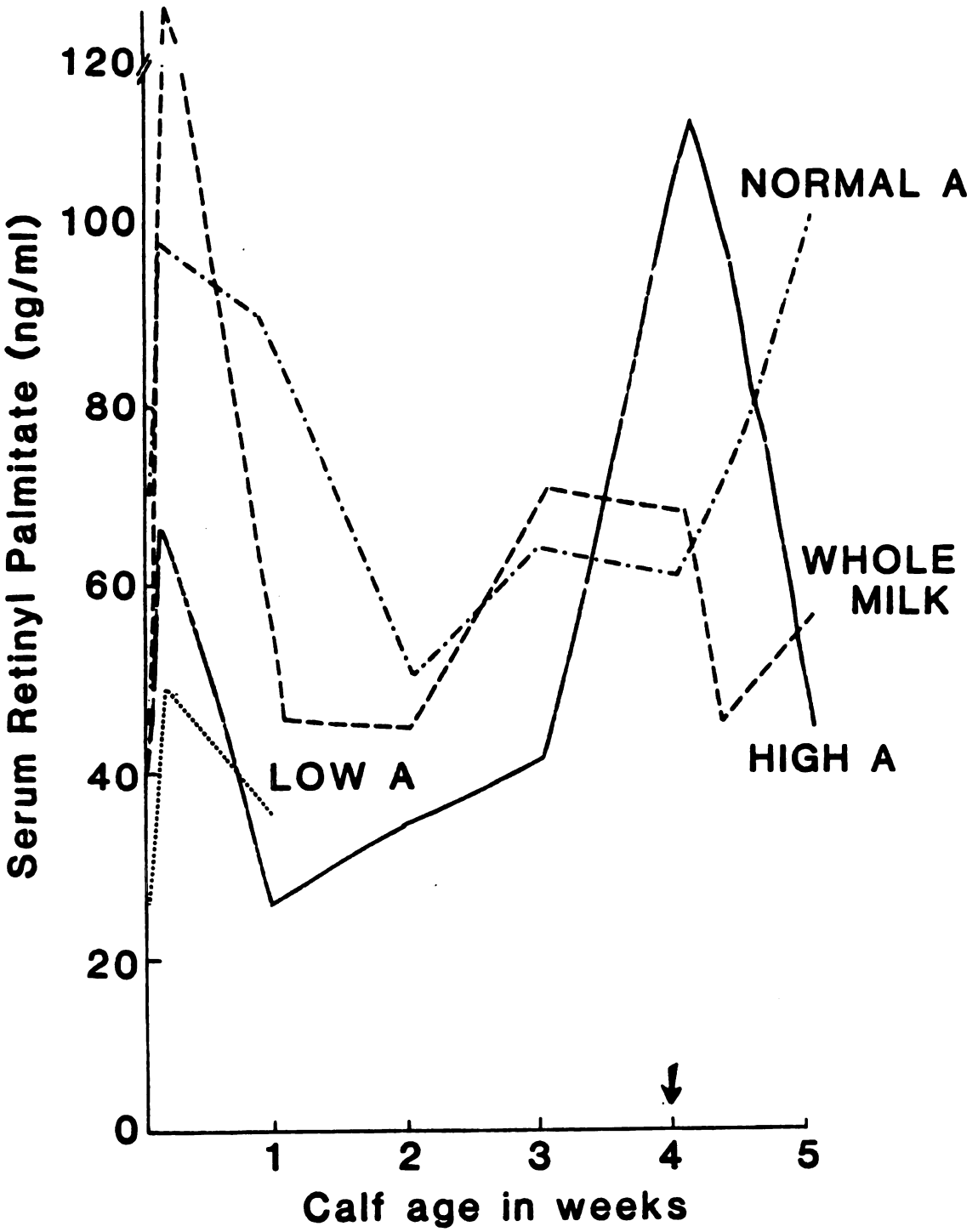
120
100
80
60
40
20
0
Serum Retinyl Palmitate (ng/ml)
NORMAL A
WHOLE MILK
HIGH A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 4

Figure 5

Serum retinyl palmitate concentractions in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

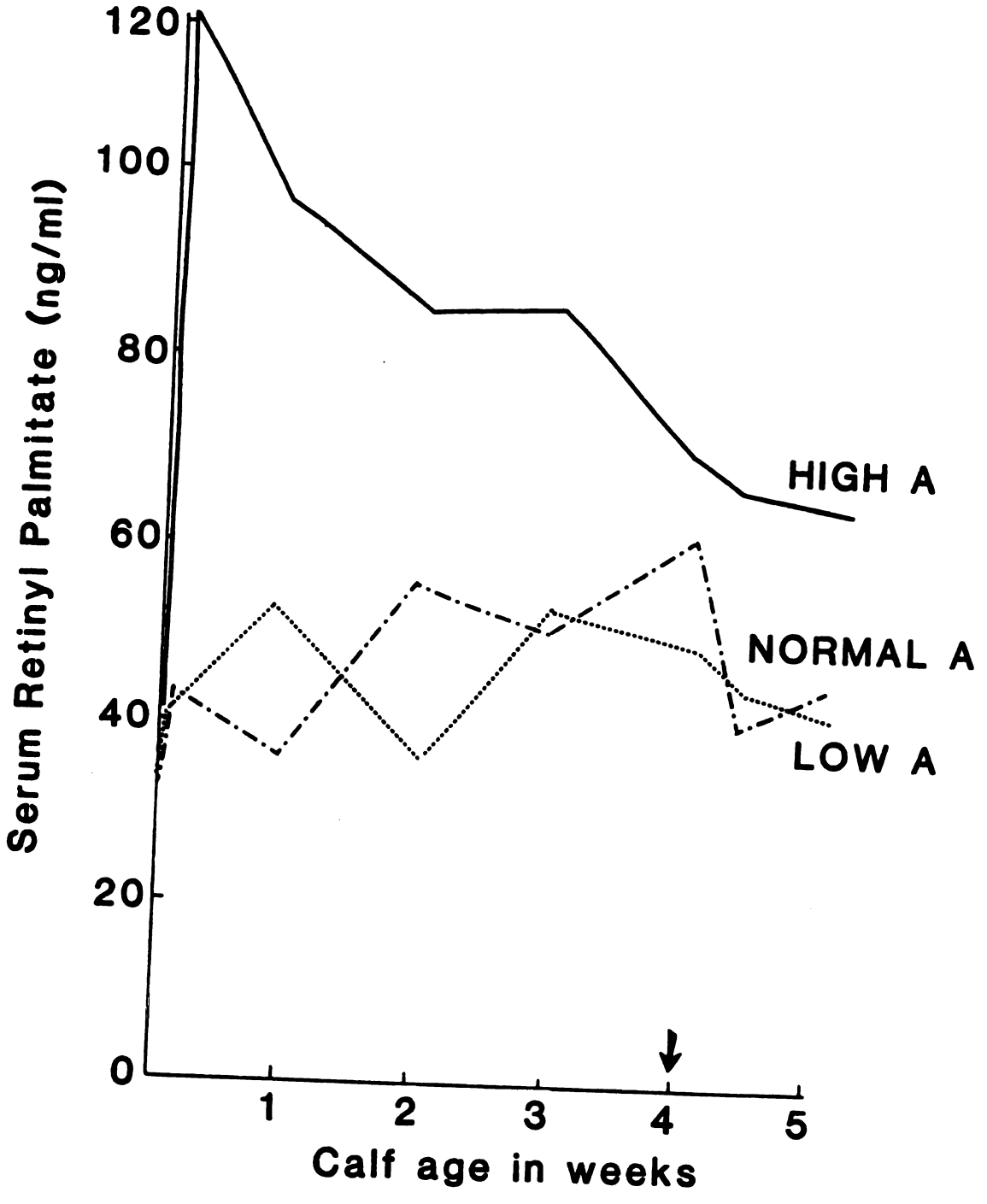
120
100
80
60
40
20
0
Serum Retinyl Palmitate (ng/ml)
HIGH A
NORMAL A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 5

at birtn (Bush and Staley 1980) and the LA calves received frozen colostrum and no supplemental vitamin A (inherent vitamin A content of milk and fat supplement was approximately 10,000 IU). After one week of age, the serum rp concentrations in Experiment 1 varied greatly throughout the experiment and showed no apparent relationship to dietary vitamin A supplementation or brucella vaccination. The fact that the HA group of calves had a lower serum rp concentration than the NA group of calves may be explained by the increased stress and hence, the increased vitamin A requirements that the HA group experienced. Also the increased health problems, (i.e., scours) experienced by the HA group of calves may have decreased absorption of the retinyl palmitate supplement (Radostitis and Bell 1970) or increased metabolic requirements (Keener et al 1942).

In Experiment 2, all groups experienced a rise in serum rp concentrations after colostral ingestion. The highest concentration was seen in the HA group and serum rp concentrations remained consistently higher in the HA group than the other two groups throughout the experiment. The NA group had a lower postcolostral concentration than was expected and the serum rp concentration remained lower than the LA group at the one-week sample. An explanation may be the fact that the NA group calves had diarrhea for the first week of life, and hence, possibly lower dietary retinyl

Figure 6

Serum retinol concentrations of calves fed milk replacer with low, normal and high vitamin A content or whole milk. These data are from calves housed in calf hutches during the winter of 1979-80 (Experiment 1).

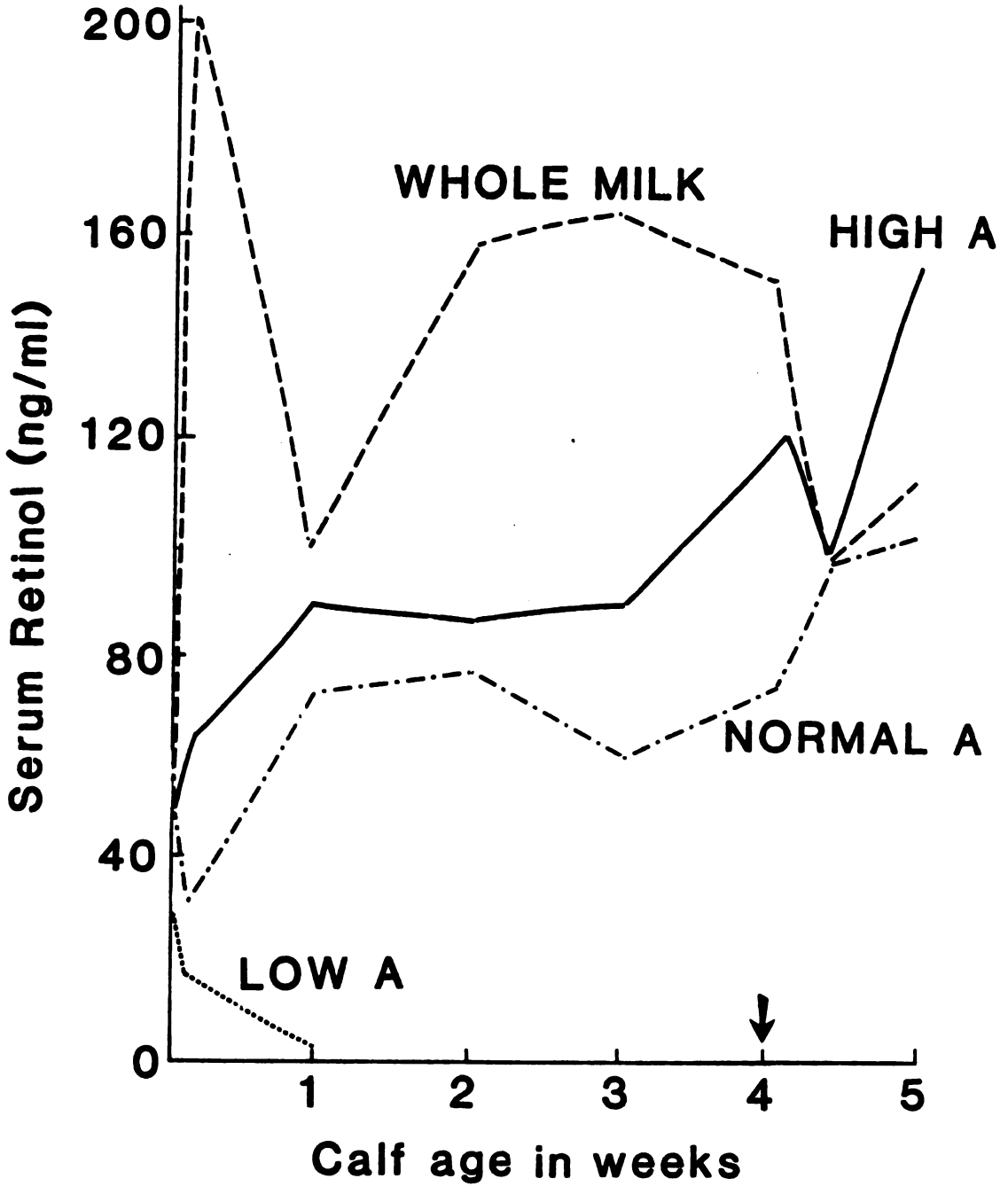
200
160
120
80
40
0
Serum Retinol (ng/ml)
WHOLE MILK
HIGH A
NORMAL A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 6

Figure 7

Serum retinol concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

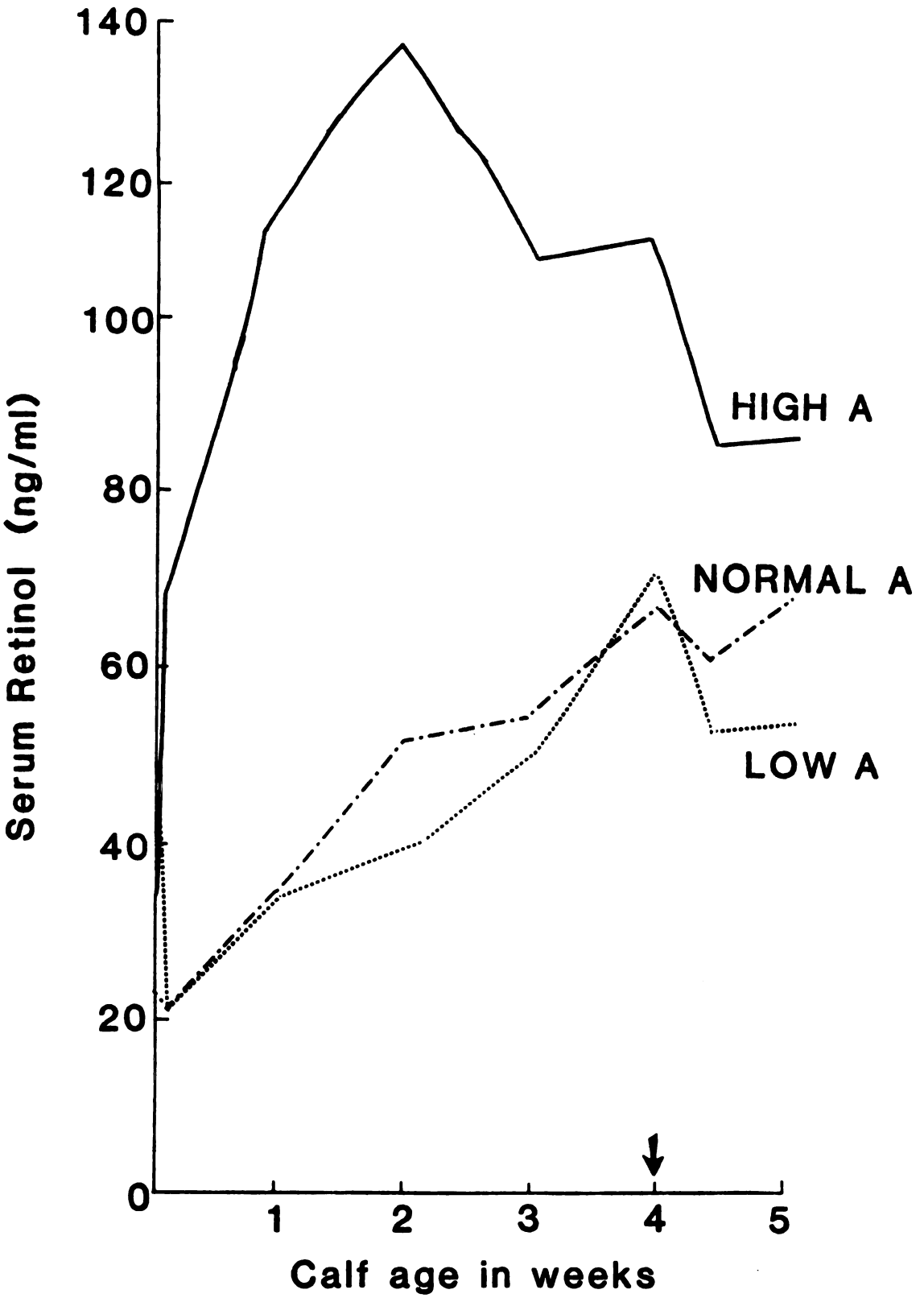
140
120
100
80
60
40
20
0
Serum Retinol (ng/ml)
HIGH A
NORMAL A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 7

palmitate absorption, increased vitamin A requirements or both. After one week, the serum rp concentrations remained similar for the LA and NA groups. No change was observed in the serum rp concentrations after brucella vaccination for any of the dietary groups.

Serum retinol

In Experiment 1, serum retinol concentrations (Figure 6) were the highest in the WM group and remained higher than the NA and HA groups ($P<.01$) from one week of age until brucella vaccination. The HA and NA groups had similar retinol concentrations (approximately 75 ng/ml) from birth to brucella vaccination. After brucella vaccination, the serum retinol concentrations increased markedly in the HA group.

In Experiment 2, the serum retinol concentrations for the HA group (Figure 7) were similar to the retinol concentrations for the NA and HA groups for Experiment 1. The retinol concentrations (Experimenmt 2) were significantly greater ($P<.01$) in the HA group than the LA and NA groups. The serum retinol concentrations in Experiment 2, more closely parallelled the dietary vitamin A supplementation. A possible explanation for the higher serum retinol concentrations in the NA group in Experiment 1 than Experiment 2 is that the environmental stress increased tissue vitamin A requirements and lead to an increase in the

Figure 8

Total serum vitamin A concentrations of calves fed milk replacer with low, normal and high vitamin A content of whole milk. These data are from calves housed in calf hutches during tn winter of 1979-80 (Experiment 1).

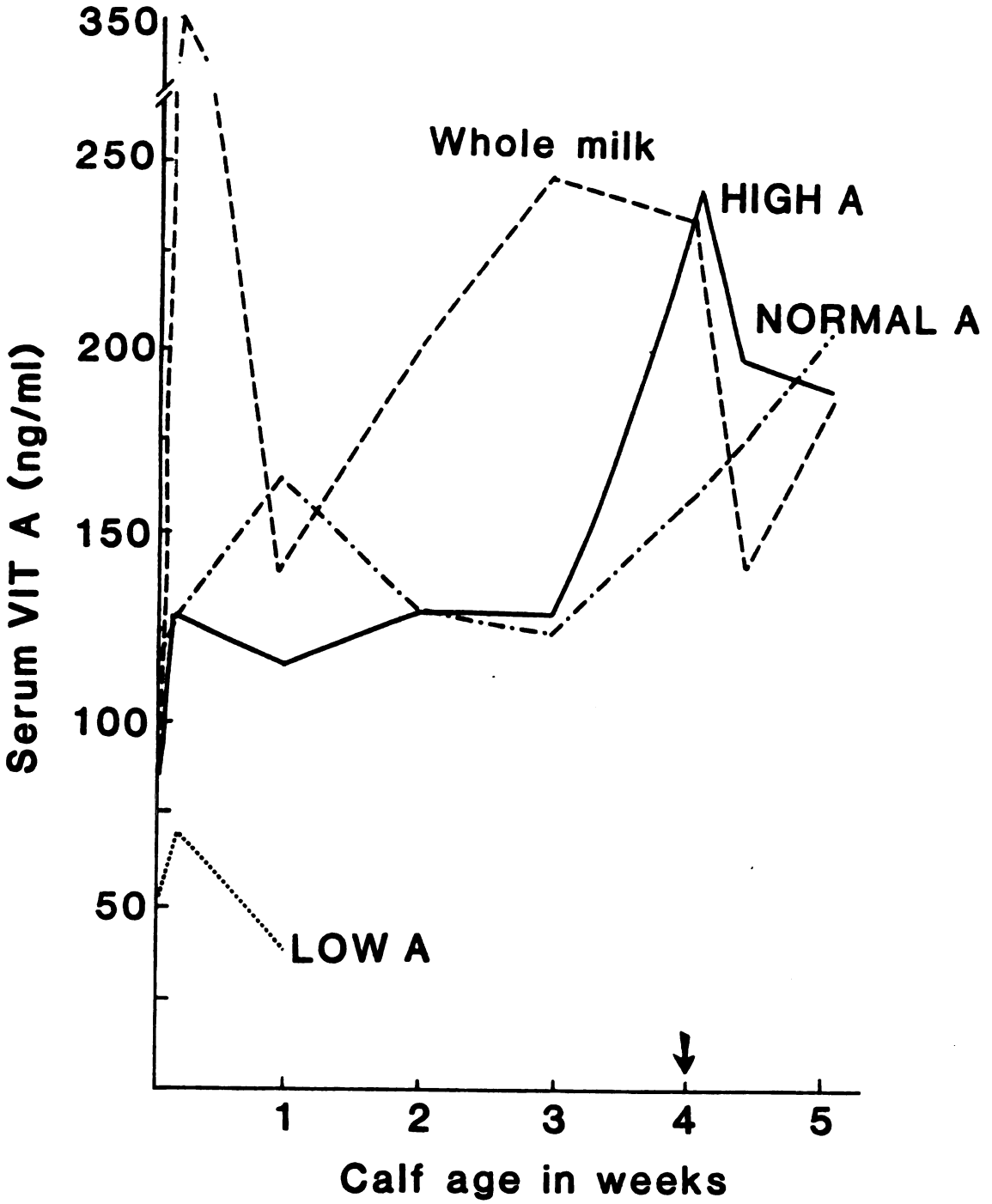
350
250
200
150
100
50
Serum VIT A (ng/ml)
Whole milk
HIGH A
NORMAL A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 8

Figure 9

Total serum vitamin A concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

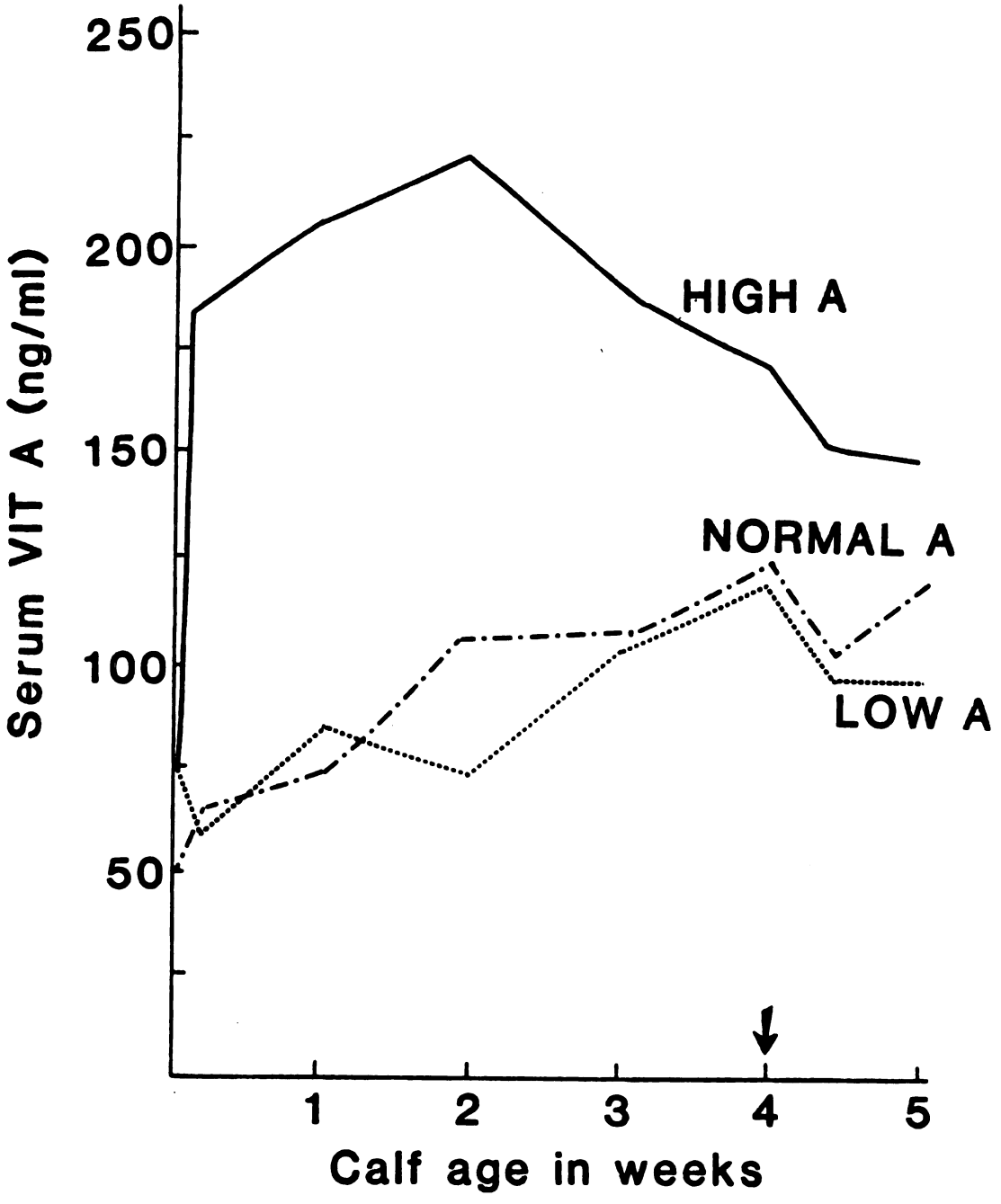
250
200
150
100
50
Serum VIT A (ng/ml)
HIGH A
NORMAL A
LOW A
1
2
3
4
5
Calf age in weeks

Figure 9

de-estrification of retinyl palmitate and increased serum retinol concentrations. However, this is not consistent with the relatively higher serum retinyl palmitate concentrations in the NA group than in the other two groups in Experiment 1. Another possibility is an increased efficiency of absorption of retinyl palmitate as a result of increased nutritional requirements.

Total Serum Vitamin A

The total serum vitamin A levels in Experiments 1 (Figure 8) and 2 (Figure 9) reflect the serum retinol concentrations more closely than the serum retinyl palmitate concentrations. All groups showed a marked increase in total serum vitamin A after colostrum ingestion in Experiment 1. In Experiment 2 the HA group showed a marked increase, the NA group a slight increase and the LA group a decrease. As previously stated, the slight increase in the NA group (Experiment 2) may be a result of the diarrhea these calves experienced at birth increasing the vitamin A requirements and/or decreasing vitamin A absorption. The lower vitamin A concentrations in the HA group (Experiment 1) as compared to the NA group (Experiment 1) and the HA group (Experiment 2) may also reflect a decrease vitamin A absorption and/or increased vitamin A requirement as a result of the digestive and respiratory disease these calves experienced.

Figure 10

Serum IgA concentrations in calves fed milk replacer with low, normal and high vitamin A content or whole milk. These data are from calves noused in calf hutches during the winter of 1979-80 (Experiment 1).

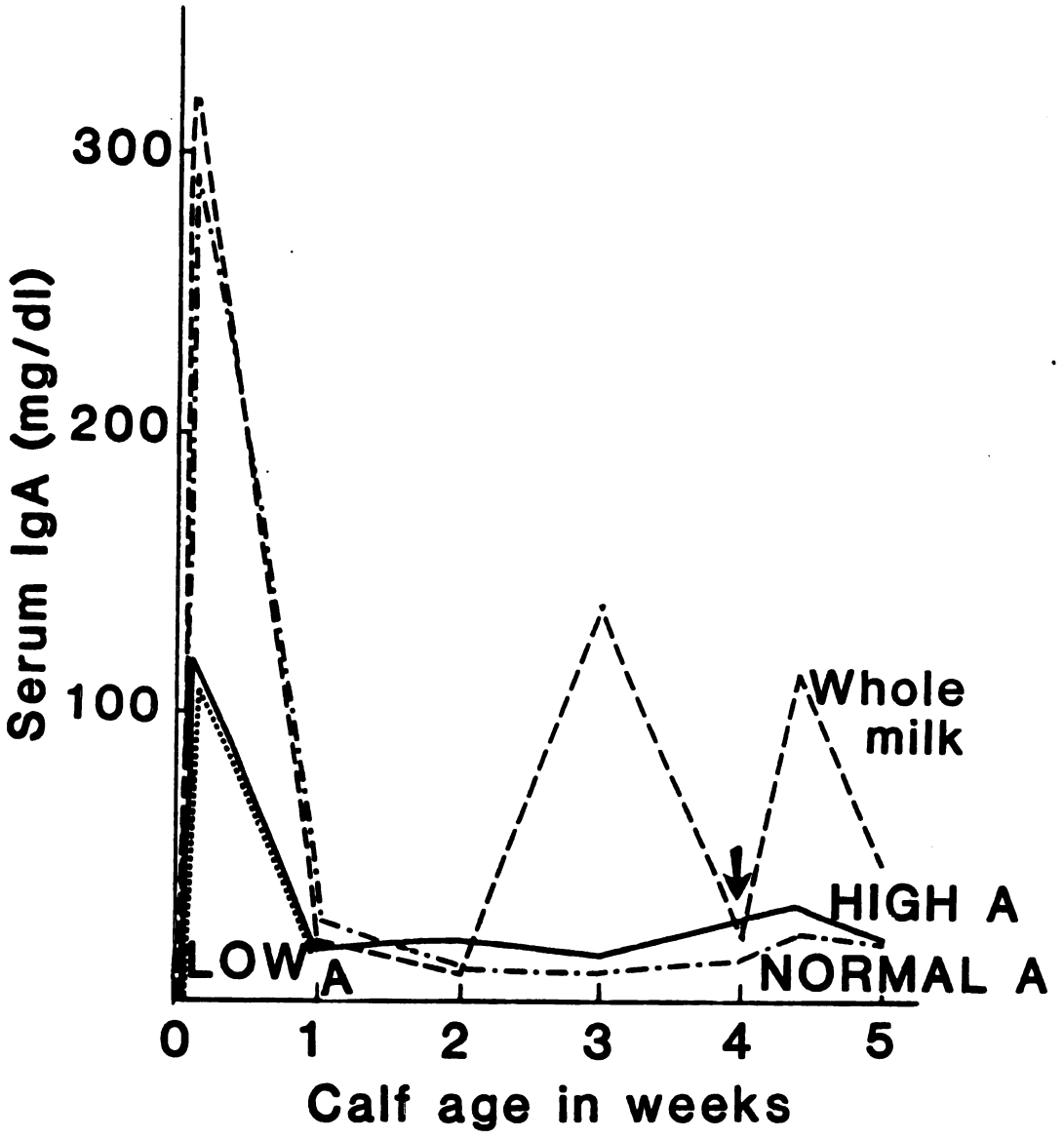
300
200
100
Serum IgA (mg/dl)
Whole milk
HIGH A
NORMAL A
LOW A
0
1
2
3
4
5
Calf age in weeks

Figure 10

Figure 11

Serum IgA concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

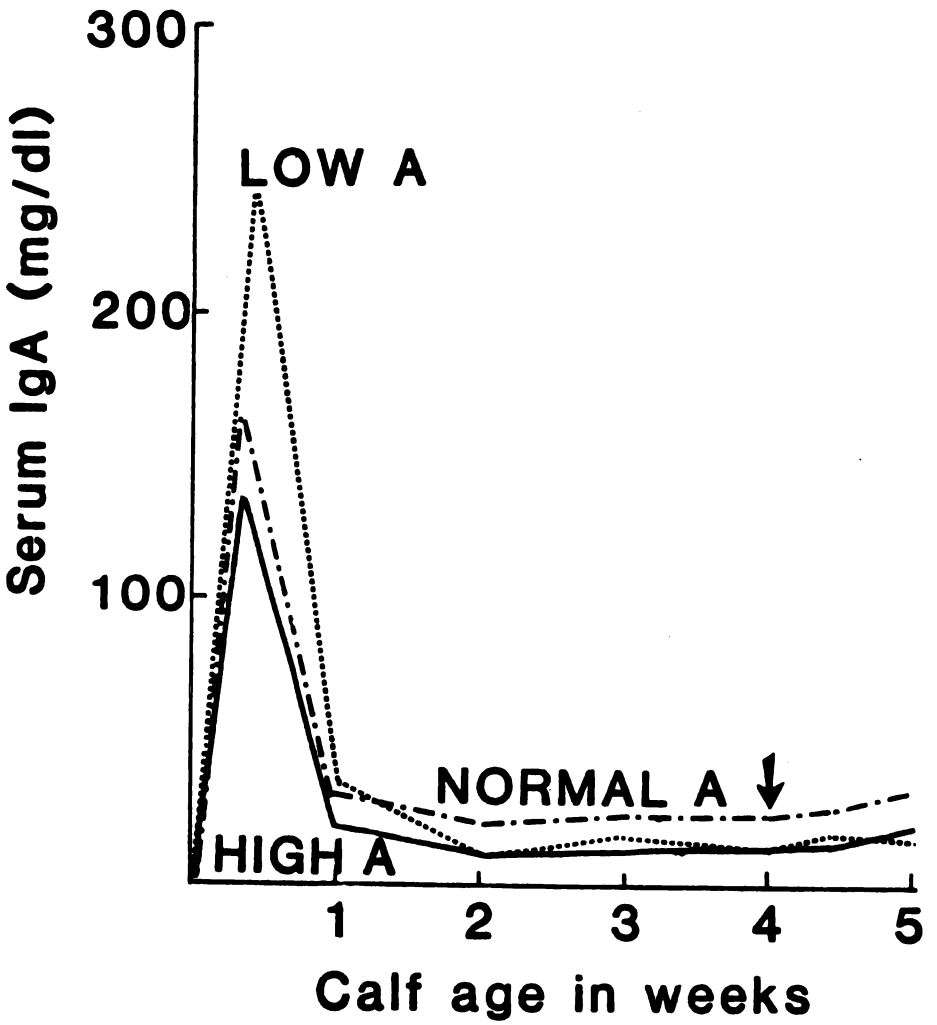
300
200
100
Serum IgA (mg/dl)
LOW A
NORMAL A
HIGH A
1
2
3
4
5
Calf age in weeks

Figure 11

In Experiment 2, the dietary supplementation more closely parallelled the total serum vitamin A concentrations. There was a significantly higher ($P<.01$) serum vitamin A concentration in the HA group from one week of age until the end of the experiment. No change was observed in either experiment after brucellosis vaccination.

IMMUNOGLOBULINS

IgA

Serum IgA concentrations are presented in Figures 10 and 11 for Experiments 1 and 2, respectively. All calves had a significant increase ($P<.05$) in IgA concentrations following colostrum ingestion. When considering individual treatments, the lower the vitamin A supplementation level, the higher the serum IgA concentrations were post-colostrally (Experiment 2). In Experiment 1, the same tendency occurred with NA and HA groups; however, the LA group absorbed the least colostrum and the WM group the most. By one week of age all groups had significantly decreased ($P<.05$) IgA concentrations to approximately 20-25 mg/dl. The IgA concentrations then remained low for the remainder of the study, except in the WM group. This group showed a fluctuation in IgA concentration throughout the study. No change was observed in serum IgA concentrations following brucella vaccination (Experiment 1). A

Figure 12

Serum IgM concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in calf hutches during the winter of 1979-80 (Experimennt 1).

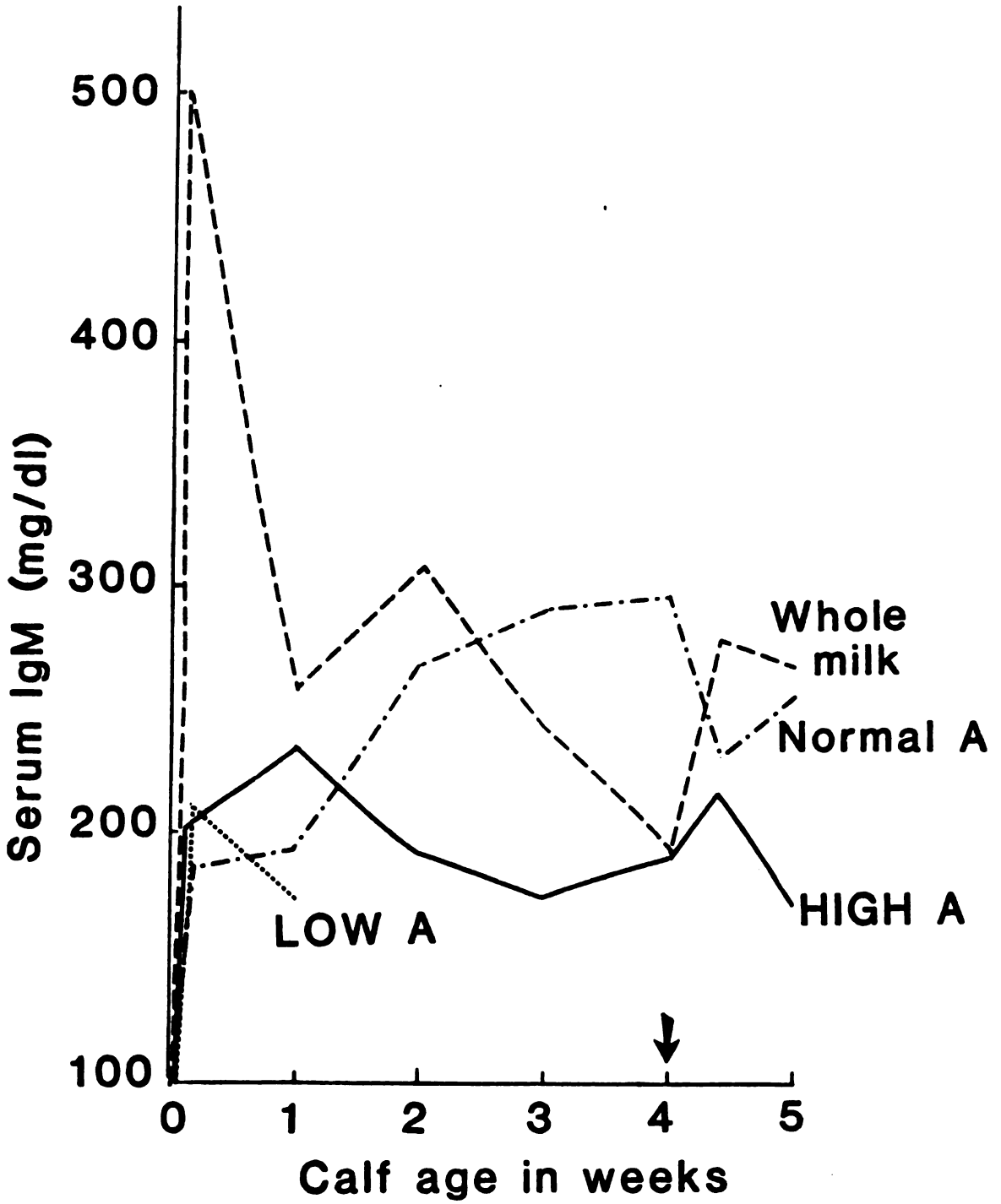
500
400
300
200
100
Serum IgM (mg/dl)
0
1
2
3
4
5
Calf age in weeks
Whole milk
Normal A
HIGH A
LOW A

Figure 12

Figure 13

Serum $_{IgM}$ concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

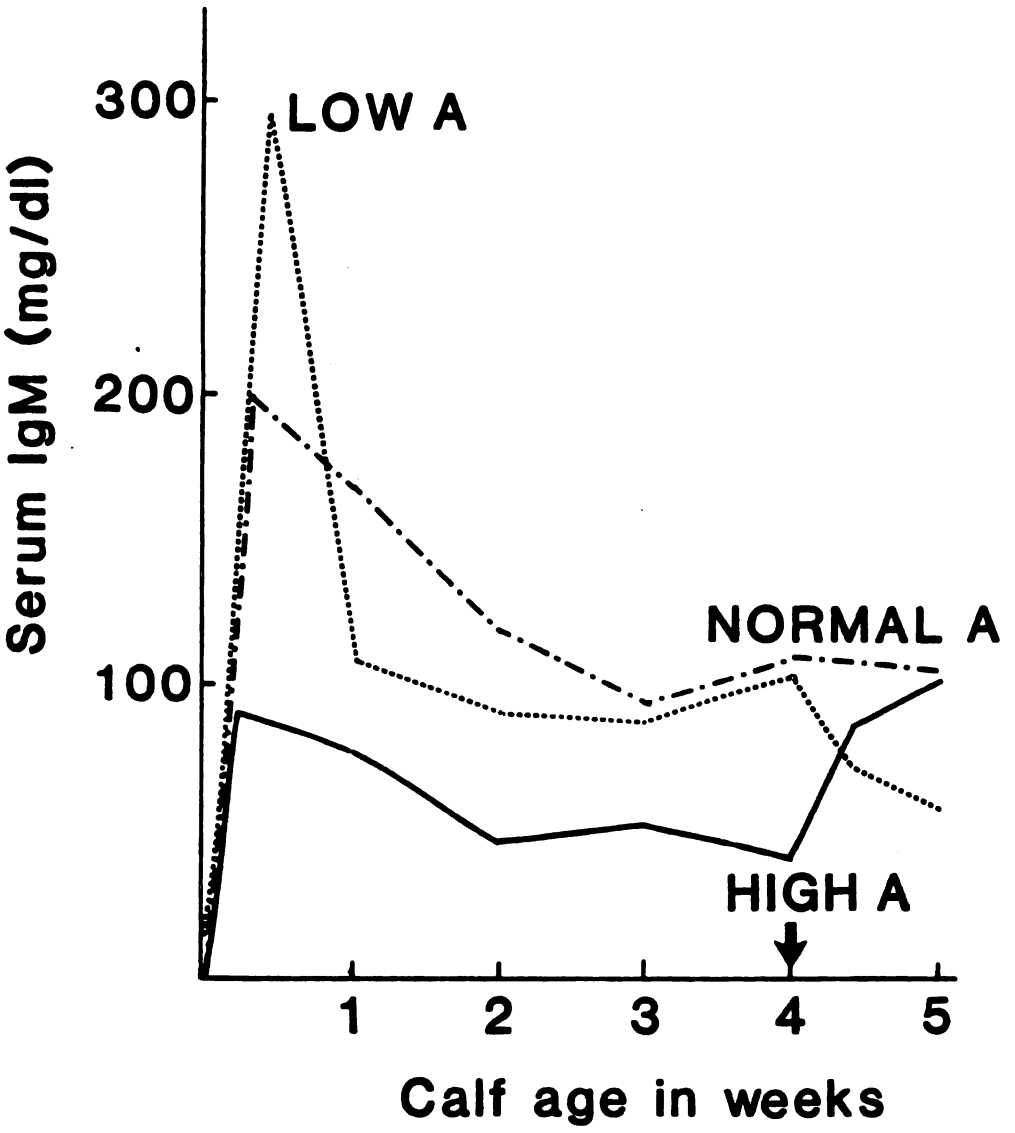
300
200
100
Serum IgM (mg/dl)
LOW A
NORMAL A
HIGH A
1
2
3
4
5
Calf age in weeks

Figure 13

significant treatment effect ($P<.05$) was seen in Experiment 2 with IgA in the NA group being higher than the other groups.

The reason for the failure of the LA group (Experiment 1) to absorb adequate colostrum, as compared to the other groups, is unknown. Possibly the calves were already experiencing enough stress that premature gut closure occurred. However, Bush (1980) showed no effect of cortisol on gut closure and Johnson (1979) stated that cortisol may prolong the absorptive period in the calf. The drop in serum IgA concentrations observed after the first week was expected since IgA is normally an Ig of mucosal surfaces and only has a life span of 2-8 days (Logan et al 1973). Also, as IgA is not a major Ig of serum and not involved in the primary humoral immune response, an increase in serum IgA concentrations following brucella vaccination would not be expected.

IgM

Serum IgM concentrations are presented in Figures 12 and 13 for Experiments 1 and 2, respectively. As with IgA, all calves showed a significant increase ($P<.05$) in serum IgM concentrations following colostrum ingestion. In Experiment 2, the groups demonstrated the same absorptive phenomenon observed with IgA; the lower the dietary vitamin A intake, the higher the postcolostral serum IgM concentrations

became. This phenomenon did not occur in Experiment 1. Following colostrum ingestion no difference was seen among the milk replacer groups with regard to serum IgM concentrations. The whole milk group absorbed more IgM than did the other groups in Experiment 1. There was a tendency for all groups to experience a decrease in serum IgM by one week of age (Experiment 2). The most marked decrease occurred in the LA group. Only the whole milk group had a marked decrease (Experiment 1); in the other groups serum IgM remained constant. For the remainder of the study, serum IgM concentrations remained relatively constant in all groups in Experiments 1 and 2; however, in the NA group in Experiment 1, there was a tendency for serum IgM concentrations to increase between one and two weeks of age.

Three days after brucella vaccination, calves in the HA and WM groups (Experiment 1) tended to exhibit a slight increase in serum IgM concentrations followed by a drop in the serum IgM seven days post vaccination. The NA group (Experiment 1) showed a decrease in serum IgM at 3 days post-vaccination, followed by a rise by seven days post-vaccination. In Experiment 2, there was a significant treatment by time interaction ($P<.05$) with the HA group showing an increase at three days post-brucella vaccination with no change at the seven-day sample period. The NA group showed no change in IgM after vaccination while the LA group

showed a marked decrease at both three and seven days post-brucella vaccination.

The fact that the NA and HA groups (Experiment 1) did not demonstrate a decrease in serum IgM concentration at one week of age, may be related to early stimulation of their immune system. Horyma (1980) and Logan (1973) have shown that calves are immunocompetent early in life. The continued increase of serum IgM in the NA group may have resulted from the continued stimulation of their immune systems. The increase in serum IgM concentrations in the HA and WM groups imnmediately following vaccination and in the NA group by three days post-vaccination (Experiment 1) may have been due to involvement of IgM in the primary humoral immune response. The decline in serum IgM concentrations in HA and WM groups (Experiment 1) and the plateau in NA and HA (Experiment 2) at seven days post-vaccination may reflect the short half life of IgM (Logan et al 1973) and an increase in IgG synthesis resulting from the continuing humoral immune response. The reason for the lack of response in the LA group (Experiment 2) is unknown. However, there was a significant positive relationship between total serum vitamin A and retinol with serum IgM in the LA group in Experiment 2. Therefore, the low dietary vitamin A may have played a role in the lack of response.

Figure 14

Serum IgG concentrations in calves fed milk replacer with low, normal and high vitamin A content or whole milk. These data are from calves housed in calf hutches during the winter of 1979-80 (Experiment 1).

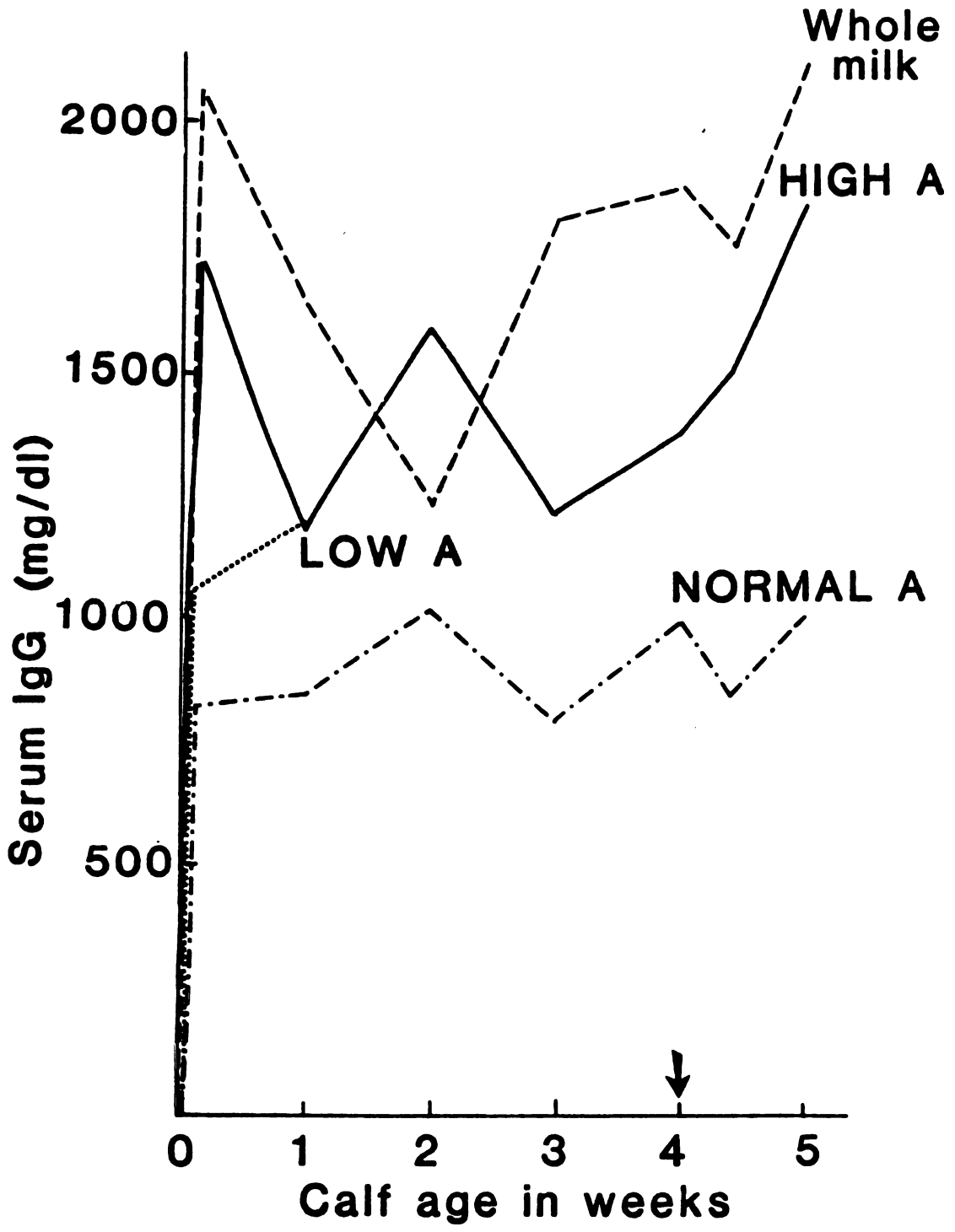
Whole milk
HIGH A
LOW A
NORMAL A
2000
1500
1000
500
Serum IgG (mg/dl)
0
1
2
3
4
5
Calf age in weeks

Figure 14

Figure 15

Serum IgG concentrations in calves fed milk replacer with low, normal and high vitamin A content. These data are from calves housed in box stalls under controlled conditions (Experiment 2).

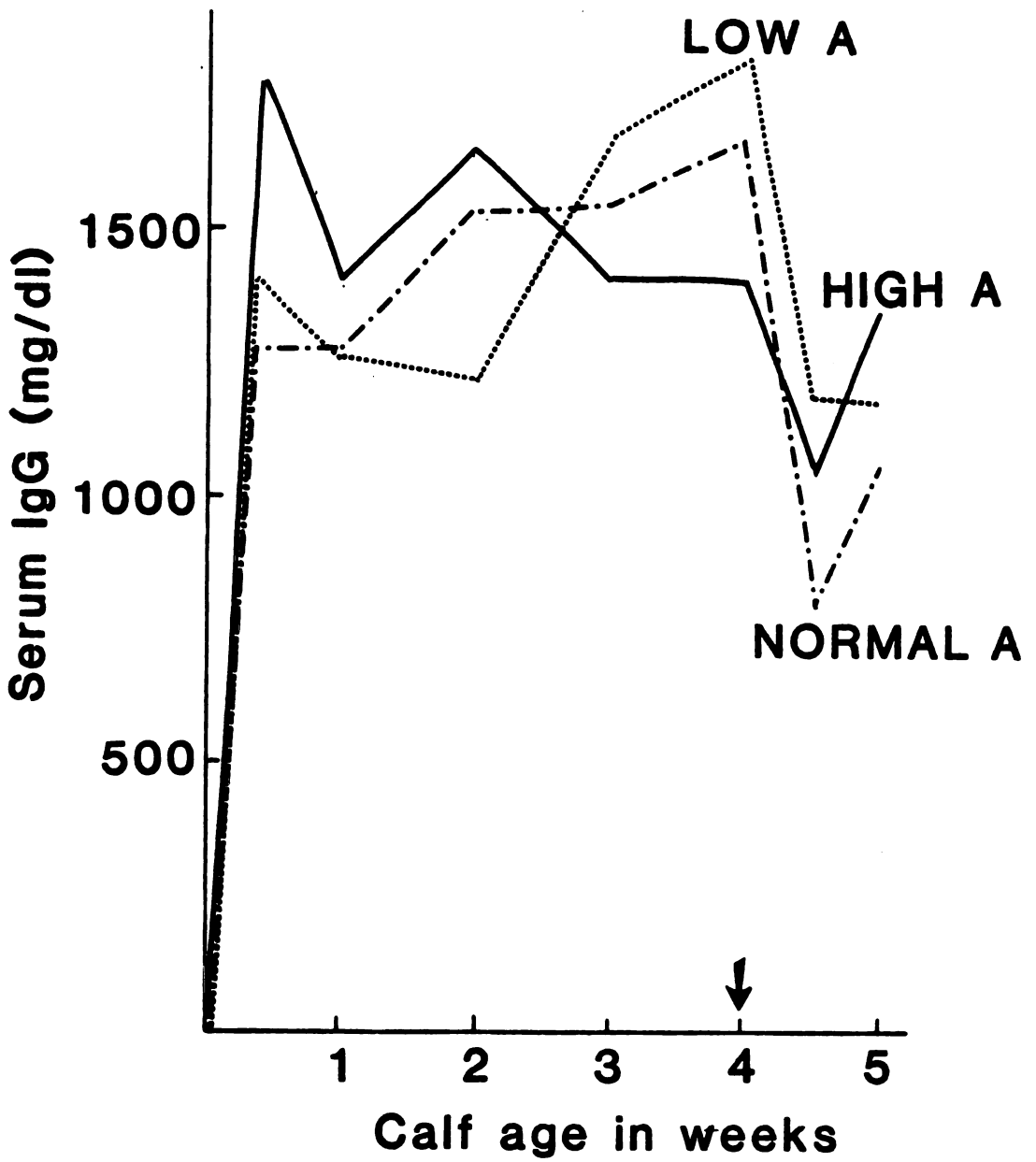
LOW A
HIGH A
NORMAL A
Serum IgG (mg/dl)
1500
1000
500
1
2
3
4
5
Calf age in weeks

Figure 15

IgG

Serum IgG concentrations are presented in Figures 14 and 15 for Experiments 1 and 2, respectively. All calves had a significant rise ($P<.05$) in IgG following colostrum ingestion. However, the greatest increase was in th HA groups (Experiments 1 and 2) and the WM group (Experiment 1). The LA and NA groups within experiments absorbed approximately the same amount of IgG. After the initial post-colostral rise, all groups experienced a slight to moderate drop in IgG followed by a plateau effect. The serum IgG then tended to increase in the LA and NA groups (Experiment 2) with time. The NA group (Experiment 1) and the HA group (Experiment 2) showed no response. At three days post-brucella vaccination, the WM and NA groups had a slight drop in serum IgG concentrations, while the HA group showed a slight increase (Experiment 1). By seven days of age, all groups exhibited an increase in serum IgG concentrations. In Experiment 2, there was a significant time interaction ($P<.025$) with all calves showing a marked decrease in serum IgG at three days post vaccination followed by an increase in the HA and NA groups and no change in the LA group by seven days post vaccination.

The increase in serum IgG over time in the WM and HA groups (Experiment 1) and the LA and NA groups (Experiment 2) may have been a result of the stimulation to their immune

systems due to concurrent health problems. It is not understood why the LA group (Experiment 1) showed no change associated with concurrent health problems. In the HA group (Experiment 2), no IgG change was observed; one explanation for the lack of IgG response was that the calves in the HA group were healthier and their immune systems may not have been stimulated. The drop in serum IgG following brucellosis vaccination observed in all groups (Experiment 2) was possibly a result of IgG reacting with brucella antigens. By seven days post-vaccination, the serum IgG concentrations in all groups (both experiments) tended to increase with the exception of the LA group (Experiment 2), thus possibly indicating a response to brucella vaccination. The reason for lack of response in the LA group (Experiment 2) is attributed to inadequate vitamin A. The delay in response may be related to the fact the IgG response is normally slower to occur than the IgM response which also has not occurred see Figures 13 and 15.

TOTAL PROTEIN

Serum total protein data are presented in Tables 5 and 6 for Experiments 1 and 2, respectively. A rise in mean serum total protein was seen in all groups (Experiments 1 and 2) after colostrum ingestion. The WM group tended to have the largest increase in total protein with the total protein in

TABLE 5

MEAN SERUM TOTAL PROTEIN OF CALVES (g/dl)--EXPERIMENT 1

	Treatment group (n=4)			
	Low[a]	Normal	High[b]	Whole[c]
Calf Age	Vit. A	Vit. A	Vit. A	Milk
Precolostrum	4.7±.2	4.1±.6	4.7±.6	4.6±.4
Postcolostrum	4.8±.6	4.6±.5	5.3±.2	6.1±.3
1week	----	5.0±.4	5.1±1.0	5.8±.5
2 weeks	----	5.0±.2	5.1±1.0	6.2±.2
3 weeks	-----	5.0±.3	4.7±.5	6.3±.3
4 weeks	----	5.3±.4	4.6±.1	6.0±.4
+3 dpv	----	5.3±.2	4.7±.1	5.8±.2
+7 dpv	----	5.4±.4	4.8±.4	6.4±.3

[a] 4 of 4 calves died by 1 week of age.

[b] 1 of 4 calves died at 1 week of age.

[c] 1 of 4 calves died before 1 week of age.

dpv = days after brucella vaccination.

TABLE 6

MEAN SERUM TOTAL PROTEIN OF CALVES (g/dl)--EXPERIMENT 2

	Treatment group (n=3)		
	Low	Normal	High
Calf Age	Vit. A	Vit. A	Vit. A
Precolostrum	4.5±.4	4.1±.3	4.1±.3
Postcolostrum	5.1±.4	4.8±.7	4.8±.3
1 week	4.8±.4	4.7±.3	5.1±.6
2 weeks	5.0±.7	5.0±.4	5.1±.2
3 weeks	5.2±.6	5.1±.2	5.0±.3
4 weeks	5.3±.5	5.1±.4	5.0±.2
+3 dpv	5.2±.7	4.7±.2	5.1±.5
+7 dpv	5.0±.3	5.3±.1	5.2±.4

dpv = days after brucella vaccination.

the milk replacer groups being lower, but consistent for all groups. This was to be expected as the WM group was fed a larger quantity of fresh colostrum while the other groups were fed a smaller quantity of pooled frozen colostrum (Bush and Staley 1980). After colostral Ig absorption, no significant changes in total protein occurred for any experimental groups.

BRUCELLA TITERS

Brucella titers were measured by the Michigan Department of Agriculture using the plate test. No change was observed in brucella titers after brucella vaccination of the calves with one-quarter dose of the vaccine. This is not unexpected as measurable titer changes do not normally occur until two to three weeks after antigenic stimulation. The one-quarter dose of brucella vaccine is believed to have provided sufficient antigenic stimulation as the State of Michigan presently uses a reduced-dose brucella vaccine which contains fewer brucella antigens than the one-quarter dose of the standard vaccine used in these experiments. Evidence of response to the vaccine was demonstrated by increases in temperature of the calves after vaccination (Appendix 5).

GENERAL

Although Butler (1963) stated colostral Ig are stable to 60 C, a major concern in the experiments was preparing a pooled colostrum which was as low as possible in vitamin A and still retained enough Ig to provide the calf with adequate passive antibody transfer. Adequate passive transfer occurred as all milk replacer groups had fairly uniform absorption of IgG, especially when calves were housed under controlled conditions. Although the colostral fat was discarded and substituted and a non-vitamin A supplemented milk replacer fed, the dietary vitamin A concentration could not be reduced below 0.5 recommended NRC vitamin A requirements. While the colostrum and milk provided more than the desired amount of vitamin A, the water-dispersible retinyl palmitate provided less vitamin A activity than anticipated. By calculations, the NA group should have received approximately 800 IU of supplemental retinyl palmitate per day; however, HPLC analysis of the retinyl palmitate revealed the calves actually obtained 500 IU of retinyl palmitate per day. A similar discrepancy between the calculated and measured dietary retinyl palmitate occurred for the HA group.

Although not intended, Experiment 1 demonstrated the relationship between environment, infection and nutrition (Scrimshaw 1977). All calves in the LA group died by one

week of age. The other groups (HA and WM) lost only one calf each, while no deaths occurred in the NA group. All calves that survived the study, however, experienced respiratory or digestive problems with the most severe problems occurring in the HA group. Keener (1942) stated that nutrient requirements double with stress and severe environment. This may have resulted in the LA calves (Experiment 1) having insufficient vitamin A for survival, while dietary vitamin A was adequate for survival in the other groups. The WM (Experiment 1) calves were the healthiest. The fact that these calves remained with their dams for 24 hours (Selman et al 1971), were fed fresh colostrum with a high Ig content (Bush et al 1980), were maintained on fresh whole milk which may have had a local mucosal protective effect (Brignole and Stott 1980) and were housed in the hutches placed in the best location may have accounted for their better health. All calves in Experiment 2 survived and did better than those in Experiment 1. Only one calf in the LA group had severe respiratory and digestive disease. In Experiment 2 dietary vitamin A levels and health of the calves were more closely related with the calves in the HA group being the healthiest.

The lack of apparent change in the serum Ig concentrations often observed during the experimnents may have resulted from a steady state between synthesis and

degradation of Ig with no net change being detectable. The effect of the antibiotic therapy on the immune system is also unknown, however, recent studies have shown chloramphenicol to decrease the immune response[18]. Following brucella vaccination and looking at the data from both Experiments 1 and 2 with multiple regression, the higher vitamin A-supplemented groups tended to exhibit a greater IgG response to brucella vaccination. This was statistically significant ($P<0.01$) when all groups were considered. If the WM group were eliminated from the data set, the significance dropped to ($P<0.1$). Hence the production of antibodies in the calf may be affected by vitamin A supplementation as is seen in rats, mice and chickens (Jurin and Tannock 1972; Wilgus 1980; Beisel 1982). However, the effect of vitamin A on the immune system tends to be very specific (Bohannon 1979). More evidence is required before a conclusion can be made. Also, these experiments made no attempt to determine the possible mechanism of the effect of vitamin A on Ig production. Bohannon (1970) and DeLuca (1969) showed a decreased synthesis of glycoprotein during vitamin A deficiency. While Jurin and Tannock (1972) suggested the effect of

---

18. N Jenkins, personal communication, Texas A and M, College Station, TX 77843.

vitamin A may be on the lysosomeme. Vitamin A has a labilizing effect on lysosomes which, in turn, may stimulate lympnoid cell division.

An experimental difference was observed for IgM production with overall production being greater in Experiment 1 than Experiment 2 after vaccination. The significant level for IgM was ($P<0.05$) with WM group included and ($P<0.1$) without the WM group included. Since IgM is the primary response to most antigenic stimuli (Beisel 1980) and all groups in Experiment 1 were subject to concurrent disease, their immune systems may have responded more rapidly than in Experiment 2 where calves tended to be healthier. After vaccination, no response was observed in IgA production with regard to treatment or experiment after vaccination. This lack of response was expected as IgA tends to be an Ig of mucosal surfaces (Sirisinha 1980). If secretory IgA production had been measured, a response may have been observed.

SUMMARY AND CONCLUSIONS

Calf mortality represents a significant loss to the cattle industry each year. The importance of colostrum and passive immunity to the calf are well documented, however, there is little known about the role other colostral components play in calf survival. Research efforts continue to minimize the calf loss within the first four weeks of life.

Two experiments involving 25 neonatal Holstein calves were conducted to determine the importance of dietary vitamin A to: 1) neonatal calf health; 2) serum vitamin A concentrations; and 3) the humoral immune status of neonatal calves. Experiment 1 was conducted with calves housed in hutches during the winter and Experiment 2 was conducted with calves housed in box stalls under controlled conditions; therefore, the effect of environmental stress was also observed. The environmental stress caused the calves in Experiment 1 to have more health problems (diarrhea, respiratory disease and death) than calves in Experiment 2. However, within each experiment, calves in the LA groups showed the most death and/or health problems. This would suggest that both vitamin A and environmental stress play a large part in neonatal calf health.

A significant relationship ($P<.01$) between serum vitamin A concentrations and dietary vitamin A supplemenation was seen in Experiment 2. However, when the calves were suffering from disease and/or other environmental stresses, there was no significant relationship between dietary and serum vitamin A levels (Experiment 1). An increased dietary vitamin A requirement during inclement conditions and disease probably accounted for this lack of correlation.

The response of the numoral immune system of the neonatal calf to different levels of dietary vitamin A was also inconsistent. As expected, no response was observed in the serum IgA concentrations since IgA is an Ig of mucosal surfaces. With regard to IgM, the HA groups tended to have a lower serum IgM concentration when compared with the LA and NA groups. The reasons for this are unclear. As the HA calves were healthier in Experiment 2 and housed under more controlled conditions, this may have resulted in less stimulation of their immune systems. However, this theory does not account for the HA calves in Experiment 1 which were being constantly exposed to immune stimuli. Another possible explanation is that vitamin A had a negative effect on IgM synthesis; however, this is unlikely because, after brucella vaccination, the IgM response tended to be greater with increasing dietary vitamin A supplementation. A further explanation is that IgM degradation may have

exceeded IgM synthesis in the HA calves (Experiment 1). Serum IgG concentrations showed no apparent relationship to vitamin A supplementation prior to brucella vaccination. After brucella vaccination, the LA group (Experiment 2) did not show a IgG response by seven days post-vaccination while the other groups had demonstrated a response.

In conclusion, there tended to be a relationship between dietary vitamin A supplementation and health, environmental conditions and serum vitamin A concentrations. Brucella vaccination appeared to increase IgM and IgG concentrations in vitamin A supplemental groups; however, no consistent statistical significance was found. Before any more definitive statements can be made with regard to the role of vitamin A in the humoral immune response of the neonatal calf, further studies need to be conducted, with more calves in each group, with different antigens used for immune stimulation, and with more extended observation periods after antigenic stimulation.

BIBLIOGRAPHY

BIBLIOGRAPHY

Alder, H. E., and A. J. Demassa. 1972. Vitamin A adjuvant with Arizona hinshawii bacterin. Appl. Microbiol. 24:849.

Allison, A. C., and L. Mallucci. 1964. Lysosomes in dividing cells with special reference to lymphocytes. T The Lancet, p. 1371.

Amstutz, H. E., T. Mull, O. M. Radostitis, R. C. Reisinger and G. B. VanNers. 1965. Symposium on infectious diarrhea of calves. Modern Vet. Pract. 46:38.

Axelrod, A. E. 1971. Immune process in vitamin deficiency states. Am. J. Clin. Nutr. 24:265.

Bach, J. F. 1976. Lymphocytes B et T. In immunologie (J. F. Bach, Ed.), Flammarion, Paris.

Bang, B. G., F. B. Bang and M. A. Foard. 1972. Lymphocyte depression induced in chickens on diets deficient in vitamin A and other components. Am. J. Path. 68:147.

Beisel, W. F. 1980. Effects of infection on nutritional status and immunity. Fed. Proc. 39:3105.

Beisel, W. F. 1982. Single nutrients and immunity. Am. J. of Clin. Nutr. 35:417

Bernier, George M. Antibody and immunoglobulin: Structure and function. 1978. In immunology II, (J. A. Bellanti, Ed.,, Saunders, PA.

Bohannon, D., T. Kiorpes anmd G. Wolf. 1979. The response of the acute phase plasma protein alpha-2 macroglobulin to vitamin A deficiency in the rat. J. Nutr. 109:1189.

Brignole, T. J. and G. H. Stott, 1980. Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. J. Dairy Sci. 63:451.

Brown, K. H., M. M. Rajan, J. Chakraborty, K. M. A. Aziz and M. Phil. 1980. Failure of a large dose of vitamin A to enhance the antibody response of tetanus toxoid in children. Am. J. Clin. Nutr. 33:212.

Bull, R. C., R. H. Ross, F. Blecha and D. P. Olson. 1974. N Nutrition and the weak calf syndronme. Departments of Animal Sciences and Veterinary Sciences, University of Idaho, Moscow, ID.

Bush, L. S. and T. G. Staley. 1980. Absorption of colostral immnunoglobulins in newborn calves. J. Dairy Sci. 63:672.

Butler, J. E. 1969. Bovine immunoglobulins: A review. J. Dairy Sci. 52:1895.

Chandra, R. K. 1972. Immunocompetence in under nutrition. 1 1972. Trop. Pediatrics 81:1194.

Chandra, R. K. 1981. Nutritional deficiency, immune responses and infectious illness. Fed. Proc. 40:3086.

Chew, B. P. and R. G. Archer. 1983. Comparative role of vitamin A and beta-carotene on reproduction and neonate survival in rats. Theriogenology 20:459.

Chytil, F. and D. E. Ong. 1978. Cellular vitamin A binding proteins. Vitamins and Hormones 36:1.

Cline, M. J. 1975. The white cell. Harvard University Press, Cambridge, Massachusetts.

Clover, C. K. and A. Zarkower. 1980. Immunologic responses in colostrum-fed and colostrum-deprived calves. Am. J. Vet. Res. 41:1002.

Cohen, B. E. and I. K. Cohen. 1973. Vitamin A adjuvant and steroid antagonist in the immune response. J. Immunol. 111:1376.

Cunningham, B. 1977. The effect of immaturity of the calf on immunological responses to strain 19 killed 45/20 adjuvant vaccines. Vet. Res. 101:238.

Cunningham, B. 1977. Protective effects of colostral antibodies to Brucella abortus strain 19 vaccination and field infection. Vet. Rec. 101:521.

DeLuca, L. and G. Wolf. 1969. Vitamin A and protein synthesis in mucous membrances. Am. J. Clin. Nutr. 22:1150.

DeLuca, L. 1977. The direct involvement of vitamin A in glycosyl transfer reactions of mammalian membranes. V Vitamins and Hormones 35:1.

Dutton, Richard. 1980. Thymphocyte subsets and interactions. Fed. Proc. 39:3109.

Eardley, Diane D. 1980. Feedback suppression. An immunoregulatory circuit. Fed. Proc. 39:3314.

Eaton, H. D. 1969. Chronic bovine hypo and hyper vitaminosis A and cerebrospinal fluid pressure. Am. J. Clin. Nutr. 22:1070.

Fleenor, W. A. and G. H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. J. Dairy Sci. 63:973.

Genstat: A general statistics program. Statistics Department, Rothamsted Experiment Station. 1977. Harpenden, Hertfordshire, Great Britain.

Goodman, D. W. 1980. Vitamin A and retinoids: Recent advances introduction, background and general overview. Fed. Proc. 39:2501.

Goodman, D. S. 1980. Vitamin A metabolism. Fed. Prod. 39:2716.

Greaves, M. F., J. J. T. Owen and M. E. Raff. 1974. T and B lymphocytes. Origins, properties and roles in immune responses. Excerpts Medica and American Elsevier Publishing Co., Inc., Amsterdam, NY.

Guilbert, H. R., C. E. Howell and G. H. Hart. 1940. Minimum vitamin A and carotene requirement of mammalian species. J. Nutr. 82:495.

Hanson, R. G., P. H. Phillips and V. R. Smith. 1946 Col Colostrum milk and its vitamin A content. J. Dairy Sci. 29:809.

Hanson, R. G., P. H. Phillips and I. W. Rupel. 1946. P Production section: Vitamins P2, P3, P5, P6, P8, P5: T The effect of vitamin supplements on survival of newborn calves. J. Dairy Sci. 29:517.

Hibbs, J. W. 1980. Meeting the vitamin A needs of dairy cattle. Ohio Report 65:67.

Horyna, B., M. Lavicka, V. Kabelik and V. Krpata. 1980. The demonstration of tne immunoglobulin levels in aborted bovine foetuses in relation to the results of histological, microbiological and virologico-serological examination. Ved. Med., Praha 25:545.

Horyna, B., and D. Draganov. 1980. The levels of colostral immunoglobulins in dead calves as related to the microbiological and patho-anatomic picture. Vet. Med., Prana 25:537.

Johnson, N. E., and W. D. Oxender. 1979. The effect of altered serum glucocorticoid concentration on the ability of tne newborn calf to absorb Ig. Am. J. Vet. Res. 40;32.

Jurin, M., and I. F. Tannock. 1972. Influence of vitamin A on immunological response. Immunology 23:283.

Keener, H. A., S. E. Bechdel, N. B. Guerrant and W. T. Thorp. 1942. Carotene in calf nutrition. J. Dairy Sci. 25:571.

Law, D. K., N. Dudrick and I. Abou. 1973. Immunocompetence of patients with protein-calorie malnutrition. Annals Int. Med. 79:545.

Leutskay, Z. I., and D. Fair. 1977. Antibody synthesis stimulated by vitamin A in chickens. Biochem. Biophys. Acta 475:207.

Logan, E. F., W. J. Penhale and R. A. Jones. 1973. Changes in the serum immunoglobulin levels of colostrum-fed calves during the first 12 weeks post partum. Res. Vet. Sci. 14:394.

Logan, E. F., A. Stennouse and D. J. Ormrod. 1974. The role of colostral immunoglobulins in intestinal immunity to enteric colibacillosis in the calf. Res. Vet. Sci. 17:290.

McGuire, T., and S. Adams. 1982. Failure of colostral immunoglobulin transfer to calves: Prevalence and diagnosis. Continuing Education 4:35.

Martin, S. W. 1975. Dairy calf mortality rate: C Characteristics of calf mortality rates in Tulare county, California. Am. J. Vet. Res. 36:1099.

Mathews, J. D., I. R. Mackay, S. Whittingham and L. A. Malcolm. 1972. Protein supplementation and enhanced antibody-producing capacity in New Guinean school children. The Lancet, p. 675.

NRC. 1976. Nutrient requiremnents of domestic animals, No. 4. Nutrient requirements of beef cattle. Fifth revised edition. National Academy of Sciences-National Research Council, Washington, D.C.

NRC. 1978. Nutrient requirements of domestic animals, No. 6. Nutrient requirements of horses. Fourth revised edition. National Academyu of Sciences-National Research Council, Washington, D.C.

Olson, D. P., A. C. S. Ward, L. F. Woodard and R. C. Bull. 1 1980. Antibody response of protein-restricted heifers to vaccination with Echerichia coli and passive transfer to their progeny. Br. Vet. J. 136:590.

Olson, J. A. 1969. Metabolism and function of vitamin A. F Fed. Proc. 28:1670.

Ong, D. E., and Chytil. 1983. Vitamin A and cancer. V Vitamins and Hormones 40:105.

Oxender, W. D., L. E. Newman and D. A. Morrow. Factors influencing dairy calf mortality in Michigan. J. Am. Vet. Med. Assoc. 162:458.

Pennale, W. J., E. F. Logan, I. E. Selman, E. W. Fisner and A. D. McEwan. 1973. Observations on the absorption of colostral immunoglobulins by the neonatal calf and their significance in colibacillosis symposium on the physiology of the neonatal calf. Ann. Res. Vet. 4:223.

Pletsityi, K. D., S. B. Vasipa, V. G. Shilinsh and M. YaYudin. 1982. Stimulation of immunity in lung cancer patients. Voprosy Pitaniia (Moskva) p. 60.

Pletsityi, D. E., S. B. Vasina, T. V. Davydova, V. G. Shilins, and YaYudin, M. 1982. Effect of vitamin A on immunity in chronic pneumonia. Institute of Endocrinology Academy of Medical Sciences of USSR. Moscow, USSR.

Radostitis, O. M., and J. M. Bell. 1970. Nutrition of the pre-ruminant dairy calf with special reference to the digestion and absorption of nutrients: A review. Can. J. Anim. Sci. 50:405.

Reddy, V., N. Raghuramulu and C. Bhaskaram. 1976. Secretory IgA in protein-calorie malnutrion. Archives of Diseases in Childhood 51:871.

Roels, O. A. 1969. The influence of vitamins A and E on lysosomes. Lysosomes in biology and pathology 1. N North-Holland Publishing Co., Amsterdam, London.

Rollins, Franklin. Vitamin symposium, Department of Animal Science, University of Arizona, Tucson, AR.

Rundles-Cunningham, S. 1982. Effects of nutritional status on immunological function. Am. Clin. Nutr. 35:1202.

SAS User's Guide. 1979. SAS Institute, Inc., Raleigh, NC.

Schillhorn van Veen, T. 1974. Drought: Malnutrition and parasitism. Nigerian J. Anim. Prod. 1:231.

Scrimshaw, N. W. 1977. Effect of infection on nutrient requirements. Am. J. Clin. Nutr. 30:1536.

Sellmeyer, E., E.BBhettay, A. S. Truswell, O. L. Meyers and J. D. L. Hansen. 1972. Lymphocyte transformation in malnourished children. Arch. Dis. Childhood 47:429.

Selmen, E. I., A. D. McEwan and E. W. Fisher. 1971. A Absorption of imnmnune lactoglobulin by newborn dairy calves. Res. Vet. Sci. 12:205.

Selman, I. E., A. D. McEwan and E. W. Fisher. 1970. Serum immunoglobulin concentrations of calves left with their dams for the first two days of life. J. Comp. Path. 80:419.

Smith, A. N., and D. G. Ingram. 1965. Immunological response of young animals. II. Antibody production in calves. Can. Vet. 6:226.

Stott, G. 1980. Immunoglobulin absorption of calf neonates with special considerations of stress. J. Dairy Sci. 63:681.

Stott, G. H., D. B. Marx, B. E. Menefee and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. 1. Period of absorption. J. Dairy Sci. 62:1632.

Stott, G. H., D. B. Marx, B. E. Menefee and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. III. Amount of absorption. J. Dairy Sci. 62:1902.

Stott, G. H., D. B. Marx, B. E. Menefee and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. IV. Effect of suckling. J. Dairy Sci. 62:1908.

Stowe, H. 1982. Vitamin A profiles of equine serum and milk. J. Anim. Sci. 54:76.

Suskind, R. M. 1977. Malnutrition and the immune response. New York Raven Press.

Suskind, R., S. Sirishinha, V. Vithayasai, R. Edelman, D. Damrongsak, C. Charupatana and R. Olson. 1976. I Immunoglobulins and antibody response in children with protein-calorie malnutrition. Am. J. Clin Nutr. 29:836.

Swain, L. 1980. Association of Ly phenotypes, T cell function and MHC recognition. Fed. Proc. 39:3110.

Tengerdy, R. P., and J. D. Brown. 1977. Effects of vitamin E and A on humoral immunity and phagocytosis on E. coli infected chickens. Poultry Sci. 56:957.

Vajda, V., and L. Slanina. 1980. Dynamics of immunoglobulins of calves in the industrial rearing system. Vet. Med.., Praha 25:527.

Vasudevan, B. and B. Dutt. 1969. Clinical syndromes in experimental vitamin A deficient calves. The Indian Vet. J. 46:658.

Wald, N., M. Idle and J. Borcham. 1980. Low serum vitamin A and subsequent risk of cancer. Lancet, p. 813.

Wilgus, H. S. 1980. Disease, nutrition-interaction. P Poultry Sci. 59:772.

Williams, W. J., E. Beutler, A. Ersler and R. W. Rundles. 1 1977. Hematology, 2nd Ed., McGraw-Hill Book Co., Blakston Publishing.

APPENDICES

APPENDIX 1

FEEDING SCHEDULE

	A		B		C	
	≤44 kg		45-50 kg		>50 kg	
Day	CMR (kg/day)	Water (liters)	CMR (kg/day)	Water (liters)	CMR (kg/day)	Water (liters)
1	.27	2.45	.32	2.86	.36	3.27
2	.27	2.45	.32	2.86	.36	3.27
3	.32	2.86	.36	3.27	.41	3.86
4	.32	2.86	.41	3.68	.45	4.09
5	.36	3.27	.45	4.09	.55	4.91
6	.41	3.68	.50	4.50	.59	5.32
7	.45	4.09	.50	4.50	.59	5.32
	<42 kg		38-48 kg		>49 kg	
8	.49	3.60	.55	4.00	.65	4.80
9	.55	4.00	.60	4.40	.65	4.80
10	.55	4.00	.65	4.80	.71	5.20
11	.60	4.40	.65	4.80	.76	5.60
12	.65	4.80	.71	5.20	.76	5.60
13	.65	4.80	.76	5.60	.82	6.00
14	.71	5.20	.76	5.60	.82	6.00
	<44 kg		45-50 kg		>50 kg	
15	.76	4.69	.83	5.08	.89	5.47
16	.76	4.69	.83	5.08	.95	5.86
17	.83	5.08	.89	5.47	.95	5.86
18	.83	5.08	.89	5.47	.95	5.86
19	.89	5.47	.95	5.86	1.02	6.25
20	.89	5.47	.95	5.86	1.02	6.25
21	.89	5.47	.95	5.86	1.02	6.25
	<46 kg		47-52 kg		>53kg	
22	.95	4.96	1.02	5.35	1.09	5.73
23	1.02	5.35	1.09	5.73	1.16	5.73
24	1.02	5.35	1.09	5.73	1.16	6.11
25	1.09	5.73	1.16	6.11	1.24	6.49
26	1.09	5.73	1.16	6.11	1.24	6.49
27	1.16	6.11	1.24	6.49	1.31	6.87
28	1.16	6.11	1.24	6.49	1.31	6.87

APPENDIX 2

LAND O'LAKES CALF MILK REPLACER

Crude Protein,not less than 24%

Crude Fat, not less than 20%

Crude Fiber, not more than 0.15

Ingredients: Dried milk, dried whey, dried whey products, dried milk protein, animal fat preserved with BHA and citric acid, lecithin, D-activated animal sterol-source of D_3, vitaminE supplement, thiamine, pyrodoxine HCL, folic acid, vitamin B_{12} supplement, choline chloride, Na silico aluminate, $MgSO_4$, ferrous SO_4, $CuSO_4$, $CoSO_4$, ethylenediamine dihydriodide and Na selenite.

Manufactured by Land O' Lakes, Inc., Agricultural Services, Fort Dodge, Iowa 50501.

APPENDIX 3

FAT SUPPLEMENT

LAND O'LAKES FM 7-40-60

Crude Protein, not less than 7.0%

Crude Fat, not less than 40.0%

Crude Fiber, not more than 0.25%

Ingredients: Dried whey, animal fat (with BHA, propyl gallate and citric acid as preservatives and propylene glycol as an emulsifier) and lecithin (a stabilizer).

Manufactured by Land O' Lakes, Inc., Agricultural Services, Fort Dodge, Iowa 50501.

APPENDIX 4

Daily weather conditions reported by the Lansing Meteorological Station, winter 1979-80, Experiment 1, Michigan State University dairy calves housed in hutches.

Date	Temp F (X)	Wind	Comments
11/26/79	48	38 SW	Fog . Rain
11/27/79	37	30 SW	Fog . Rain . Haze
11/28/79	28	16 W	Fog
11/29/79	26	21 W	Fog . Haze . Snow
11/30/79	25	21 W	
12/1/79	23	18 W	Fog . Snow . Haze Blowing
12/2/79	14	15 NW	
12/3/79	23	27 SW	
12/4/79	28	19 S	Fog
12/5/79	42	32 SW	
12/6/79	35	14 W	
12/7/79	37	30 SW	Fog . Rain
12/8/79	25	23 NW	
12/9/79	32	32 SW	Snow showers

Date	Temp F (X)	Wind	Comments
12/10/79	38	24 SW	Fog
12/11/79	52	24 SW	Fog . Drizzle
12/12/79	34	15 NW	Fog . Haze . Rain
12/13/79	25	20 NW	Fog . Snow
12/14/79	25	17 NW	
12/15/79	32	26 SW	
12/16/79	24	28 N	Fog . Freezing rain
			Blowing snow
12/17/79	10	25 W	Haze
12/18/79	19	13 S	
12/19/79	30	12 S	
12/20/79	25	17 SE	Fog . Haze . Drizzle
12/21/79	30	18 S	Fog . Haze . Drizzle
12/22/79	41	17 S	Fog . Rain
12/23/79	47	15 S	Fog . Drizzle
12/24/79	44	27 NE	Fog . Rain
12/25/79	34	30 NE	Fog . Rain . Sleet
12/26/79	30	14 W	Fog . Drizzle
12/27/79	30	17 W	Fog
12/28/79	36	17 W	Fog
12/29/79	36	15 W	Fog
12/30/79	29	8 W	Fog
12/31/79	27	5 W	Fog

Date	Temp F (X)	Wind	Comments
1/1/80	27	10 W 24	Fog
1/2/80	28	13 W 05	Fog . Freezing drizzle Snow
1/3/80	23	12 W 04	
1/4/80	21	14 W 06	
1/5/80	22	15 W 04	
1/6/80	19	30 W 25	Blowing snow
1/7/80	25	38 W 25	Blowing snow
1/8/80	15	23 W 28	Blowing snow . Snow snowers
1/9/80	8	15 W 27	Snow
1/10/80	24	25 W 16	Fog . Haze
1/11/80	34	38 W 28	Fog . Rain . Snow
1/12/80	14	29 W 26	Blowing snow
1/13/80	31	24 W 18	Sleet
1/14/80	31	17 W 23	Fog . Haze
1/15/80	32	17 W 14	Fog
1/16/80	39	23 W 15	Fog . Rain . Haze
1/17/80	42	18 W 23	Fog . Rain . Drizzle
1/18/80	33	17 W 28	
1/19/80	30	17 W 28	
1/20/80	26	20 W 26	
1/21/80	29	14 W 27	Snow

Date	Temp F (X)	Wind	Comments
1/22/80	27	17 W 23	Fog . Snow
1/23/80	12	22 W 26	Snow showers
1/24/80	12	13 W 14	Snow
1/25/80	14	13 W 34	
1/26/80	12	12 W 25	Fog . Snow
1/27/80	17	15 W 23	Snow showers
1/28/80	14	14 W 28	Snow
1/29/80	14	20 W 28	Snow showers
1/30/80	9	12 W 29	
1/31/80	8	10 W 01	
2/1/80	8	12 W 01	
2/2/80	13	9 W 02	
2/3/80	8	18 W 35	
2/4/80	13	12 W 31	
2/5/80	13	12 W 09	Snow
2/6/80	21	15 W 05	Fog . Snow
2/7/80	19	10 W 01	Snow
2/8/80	16	7 W 36	
2/9/80	24	8 W 34	Fog . Snow
2/10/80	17	20 W 21	Fog . Snow

APPENDIX 5

Temperature of calves after Brucella Vaccination

Calf	Days after vaccination							
No.	0	1	2	3	4	5	6	7
LA-1	A	A	106.0	104.4	104.0	104.4	103.2	103.0
	A	A	105.6	104.6	104.2	104.1	103.4	A
LA-2	102.4	101.2	102.6	103.6	102.6	103.4	104.0	103.8
	103.0	103.2	103.4	104.0	104.2	A	103.0	104.6
LA-3	101.2	103.4	104.6	103.0	101.8	101.0	102.4	101.8
	A	102.2	105.8	103.4	102.0	103.0	101.8	A
NA-1	101.8	104.0	104.8	A	A	A	A	A
	105.4	104.7	104.8	A	A	A	A	A
NA-2	101.4	103.0	102.8	A	A	A	A	A
	101.8	103.2	104.5	A	A	A	A	A
NA-3	102.0	103.5	102.4	A	A	A	A	A
	103.2	104.4	103.2	A	A	A	A	A

Calf	Days after vaccination							
No.	0	1	2	3	4	5	6	7
HA-1	A	A	A	A	A	A	A	A
	A	A	A	A	A	A	A	A
HA-2	102.2	105.4	103.6	105.4	101.2	101.6	101.2	102.6
	104.2	102.4	105.4	A	102.4	102.3	102.4	A
HA-3	102.6	106.0	102.8	105.4	102.8	101.2	101.8	102.4
	104.0	103.3	104.8	103.2	102.4	103.0	103.0	A

Both AM and PM temperatures were recorded.

A = No temperature Taken.

LA-2 calf was treated with antibiotics for the entire period after vaccination.